METHODS FOR MODULATING
FXR RECEPTOR ACTIVITY

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This application claims priority under 35 USC 119(e) to Provisional Patent Application Serial No. 60/138,968, filed June 11, 1999, hereby incorporated by reference herein.

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Field of the Invention

The present invention is relevant to the fields of human and veterinary
15   medicine, physiology and biochemistry, particularly in the regulation of lipid metabolism and catabolism and cholesterol synthesis and breakdown.

Background of the Invention

20   A vast array of specific metabolic, developmental, and catabolic processes appear to be directly or indirectly regulated *in vivo* by comparatively small molecules such as steroids, retinoids and thyroid hormones. The mechanism whereby a single such compound can contribute to the regulation of numerous different cellular events was the subject of much speculation until relatively
25   recently, when it was discovered that these compounds each share the ability to bind to transcriptionally active proteinaceous receptors. These protein receptors, in turn, are able to bind specific cis-acting nucleic acid regulatory sequence regions, termed response elements or RE's, located upstream of the coding sequence of certain genes and to activate the transcription of these genes. Thus, the
30   proteinaceous receptors can serve as specific, ligand–dependent regulators of gene transcription and expression.

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The amino acid sequences of these various receptors were quickly found to share regions of homology, thus making each such receptor a member of a family of ligand-modulated receptor molecules. This family has been termed the steroid superfamily of nuclear hormone receptors; nuclear, because the receptors are

5   usually found in high concentration in the nucleus of the cell.

Further study of the structural and functional relationship between the nuclear hormone receptors has shown certain characteristics in common between them in addition to sequence homology. *See e.g.,* Evans et al. *Science* 240:889-895 (1988). As stated above, the nuclear hormone receptors are able to bind to *cis-*

10   acting regulatory elements present in the promoters of the target genes. The glucocorticoid, estrogen, androgen, progestin, and mineralcorticoid receptors have been found to bind as homodimers to specific response elements organized as inverted repeats.

Another class of nuclear hormone receptors, which includes the retinoid

15   receptor RAR (retinoic acid receptor), the thyroid receptor, the vitamin D receptor, the peroxisome proliferator receptor, and the insect ecdysone receptor bind their response element as a heterodimer in conjunction with the retinoid X receptor (RXR), which in turn is positively activated by 9-cis retinoic acid. See Mangelsdorf, et al., *The Retinoid Receptors* in *The Retinoids: Biology, Chemistry*

20   *and Medicine* Ch.8 (Sporn et al., eds. 2d ed., Raven Press Ltd. 1994); Nagpal and Chandraratna, *Current Pharm. Design* 2:295-316 (1996), which are both incorporated by reference herein. The retinoid receptors RAR and RXR, like many nuclear receptors, exist as a number of subtypes (RARα, RARβ, RARγ, and RXRα, RXRβ, and RXRγ). Additionally, each subtype may exist in different

25   isoforms.

While the nuclear hormone receptors referenced above have all been shown to have specific ligand partners, nucleic acid and amino acid sequencing experiments and sequence alignment and comparison have revealed a class of protein molecules retaining significant sequence homology and structural similarity

to the nuclear hormone receptor superfamily, but for which no corresponding ligand has yet been discovered. In fact, some of these "receptors" have been discovered to require no ligand binding to exhibit transcriptional activity. These receptors have been collectively termed "orphan" receptors.

5   Products of intermediate metabolism are known transcriptional regulators in prokaryotes and lower eukaryotes such as yeast; thus there has been speculation that such metabolites may also serve this function in higher organisms, perhaps through interaction with the nuclear hormone receptors.

Farnesol is an isoprenoid involved in the mevalonate biosynthetic pathway,
10   which leads to the synthesis of cholesterol, bile acids, porphyrin, dolichol, ubiquinone, carotenoids, retinoids, vitamin D, seroid hormones, and farnesylated proteins. Farnesyl pyrophosphate, a derivative of farnesol, is the last common intermediate in the mevalonate biosynthetic pathway.

Foreman et al., *Cell* 81:687-693 (1995) have demonstrated that an orphan
15   receptor, now termed farnesoid X-activated receptor (FXR), is activated by farnesol and related molecules. This reference is hereby incorporated by reference herein. FXR is expressed in the liver, gut, adrenal gland, and kidney.

The amino acid sequence of FXR reveals a conserved DNA-binding domain (DBD) and ligand-binding domain (LBD). The LBD comprises subdomains
20   responsible for ligand binding, receptor dimerization, and transactivation. Additionally, cells expressing chimeric proteins that contain the LBD of FXR fused to the DBD of the yeast GAL4 transcription activator did not transcribe a reporter gene containing a GAL4 response element unless the FXR construct was coexpressed with another protein comprising the dimerization and ligand binding
25   subdomains of RXR. These data suggested that FXR and RXR interact to form a transcriptionally active dimer. No interaction was seen between FXR and any other nuclear hormone receptors that were tested. *Id.*

Among the nuclear hormone receptors amino acid sequence homology to FXR is high in the insect ecdysone receptor (EcR), which dimerizes with an RXR

homolog. When dimerized with RXRα, FXR was shown to specifically bind hsp27, an EcR response element, however, binding was not seen when FXR was expressed alone. FXR and RXR bind to certain sequences as a heterodimer.

The FXR-RXRα complex was found to be activated by juvenile hormone
5   III (JH III); incubation of cells transfected with RXR and FXR. The cells were also transfected with a reporter plasmid containing 5 copies of the hsp27 response element within a portion of the mouse mammary tumor virus (MTV) promoter; the promoter was positioned upstream of the firefly luciferase gene. Activation of this gene results in the expression of luciferase, which is easily quantifiable as a
10   measure of transactivation activity. Other potential ligands, including selected steriods, and eicosanoids were found to have no effect in this system. JH III failed to activate other nuclear hormone receptors, and does not activate either FXR or RXR alone. Forman et al., *Cell* 81:687-693 (1995).

JH III is a derivative of farnesyl pyrophosphate. Other farnesyl derivatives
15   have been tested for the ability to activate the FXR-RXR complex. Farnesol was demonstrated to strongly activate the heterodimer. Other derivatives such as farnesal, farnesyl acetate, farnesoic acid and geranylgeraniol activated the FXR-RXR complex somewhat less strongly; the farnesyl metabolites geraniol, squalene and cholesterol did not activate FXR-RXR. *Id.*

20   Cholesterol synthesis is closely regulated by modulation of the levels of 3-hydroxy-3-methylglutaryl-coenzyme A reductase(HMG-CoA), which regulates the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate. Through a series of phosphorylations and a decarboxylation reaction, mevalonate is converted into 3-isopentenyl pyrophosphoric acid, which isomerizes to 3,3-dimethylallyl
25   pyrophosphoric acid. An enzyme-mediated condensation reaction between the 5 carbon isoprenyl compounds 3-isopentenyl pyrophosphoric acid and 3,3-dimethylallyl pyrophosphoric acid results in the formation of the 10 carbon diisoprenyl compound geranyl pyrophosphoric acid. This, in turn, reacts with another molecule of 3-isopentenyl pyrophosphoric acid to form the 15 carbon

compound farnesyl pyrophosphate. Two molecules of this latter compound react to form the 30 carbon molecule presqualine pyrophosphate, which is dephosphorylated to form squaline. Squaline is then cyclized to form cholesterol. Thus, HMG-CoA reductase mediates the initial formation of the isoprene units that

5   are subsequently assembled in series and cyclized to form cholesterol.

The levels of HMG-CoA reductase are governed in part by controlling the gene transcription, translation, and by degradation of the enzyme. Farnesol has recently been shown to be involved in the regulation of HMG-CoA reductase degradation. Evidence exists for the synergistic promotion of HMG-CoA reductase

10   degradation by farnesol and a sterol component, such as 25-hydroxycholesterol. *See e.g.*, Meigs et al., *J. Biol. Chem.* 271:7916-7922 (1996), hereby incorporated by reference herein.

Cholesterol is the precursor of various compounds such as sterols, bile acids such as cholic acid, and the steroid hormones such as testosterone and

15   progesterone. All these compounds retain the basic cholesterol nucleus. The more polar bile acids are formed in the liver and secreted into the small intestine, where they aid in the absorption of lipids. The formation of bile acids from cholesterol is therefore an important degradation pathway for cholesterol, and is a key determinant of the steady-state concentration of cholesterol in the body.

20   The rate-limiting enzyme in the formation of bile acids from cholesterol is cholesterol 7α-hydrolase (Cyp7a). For some time it has been known that bile acids act in a negative feedback loop to limit their own production via this pathway, but the means by which this is accomplished has remained elusive. Recently, there has been evidence that Cyp7a synthesis and expression is inhibited by bile acids.

25   Chiang, *Front. Biosci.* 3:D176-93 (1998) hereby incorporated by reference herein.

Despite the fact that cholesterol is essential for the synthesis of cell membranes and various hormones and other small molecules, raised levels of cholesterol, particularly in the form of low density lipoprotein (LDL), have been strongly linked to arteriosclerosis and other cardiovascular diseases. Additionally,

maintenance of appropriate bile acid concentrations is important in regulating lipid metabolism, and may be useful in the prevention of colon cancer and gallstone formation.

Among currently available drugs for the treatment of hypercholesterolemia are ion exchange media such as colestipol and cholestyramine. These drugs function by sequestering bile acids in the gut; the bile acids are then excreted in the feces. Because the intestine does not reabsorb the sequestered bile acids, the bile acids are no longer available to inhibit the formation of bile acids by cholesterol degradation. As a result, bile acid synthesis is "depressed" with the result that the steady-state concentration of cholesterol is lowered.

Unfortunately, these ion exchange drugs have been associated with an increased incidence of intestinal tumors in rodents. Additionally, since the drugs are highly charged, they are capable of adsorbing other compounds, such as ingested drugs, naturally occurring hormones, regulatory factors and the like.

Recently a poster displayed by Neisor, Flach, Weinberger & Bentzen at an AACR conference on Nuclear Receptors in Palm Springs, California held on Jan 8-11, 1999 discussed the ability of certain 1,1-biphosphonate esters to activate FXR and to lower plasma cholesterol levels in mammals. This poster abstract is incorporated by reference herein.

Thus, there remains a need in the art for methods of modulating the steady-state concentration of cholesterol and/or bile acids. Such methods preferably do not significantly interact with other therapeutic agents, and function to help promote the breakdown or formation of cholesterol in a more direct fashion.

Brief Description of the Drawings

Fig. 1 is a dose-response curve plotting the ability of the GAL-RXR and GAL-RXR$_m$ proteins to transcriptionally activate UAS$_G$-Tk-Luc in the presence of an

5   RXR agonist, LG 268.

Fig. 2 is a graph comparing the ability of FXR-RXRα and FXR-RXR$_m$ heterodimers to transactivate a reporter gene in the presence of a bovine bile acid preparation, LG 268 and AGN 10.

Fig. 3 is a graph comparing the ability of the FXR-RXR$_m$ heterodimer to

10 transactivate a reporter gene in the presence of selected bile acids, LG 268 and AGN 10.

Fig 4 shows a dose-response curve comparing the agonist activity of selected bile acids and AGN 10.

Fig. 5a through 5d provides the structure of compounds comprising a panel of

15 prospective FXR ligands.

Fig. 6 provides a graph comparing the FXR agonist activities of the compounds of Fig. 5a-5d in the presence and absence of FXR alone, RXRα alone, a FXR-RXR heterodimer and a FXR- RXRm heterodimer.

20 ## Summary of the Invention

The present invention is directed to methods for modulating the transcriptional activity of FXR through the use of synthetic FXR ligands. In a preferred embodiment the compositions are ligands of FXR able to cause FXR, alone or preferably in combination with another nuclear hormone receptor such as

25 RXR, to suppress, inhibit, or stimulate the transcription of a given target gene. In a currently most preferred embodiment, FXR is substantially inactive in its ability to modulate gene expression unless it associates with RXR. Additionally, it is preferred that the FXR ligand is not substantially active as a modulating ligand of either or both RAR and RXR receptors.

It has been discovered that, when activated by an appropriate ligand, FXR is a bile acid receptor that is able to regulate the expression of Cyp7a, thereby controlling a key step in the degradation of cholesterol. Thus, in a particularly preferred embodiment, the invention concerns methods for controlling the

5   concentrations of cholesterol and bile acids *in vivo* through the use of specific FXR ligands. *See* Wang, et al., *Molec. Cell* 3:543-553 (May 1999), hereby incorporated by reference herein. In another embodiment, a synthetic FXR agonist may be used to increase the concentration of cholesterol within a hypocholesterolemic mammal.

Of course, ligand-dependent activities of FXR other than the regulation of

10   Cyp7a expression can also be controlled through the use of an appropriate FXR ligand. For example, also contemplated by the present invention are methods for regulating the concentration of bile acids in a mammal. A heightened concentration of bile acids in mammals has been associated with an increased occurrence of colon cancer; thus, the use of FXR ligands to lower abnormally high

15   bile acid concentrations may provide a therapeutic and/or prophylactic effect for this indication. Additionally, proteins other than Cyp7a are regulated by bile acids; these include Intestinal Bile Acid Binding Proteins and Cyclooxygenase 2 (both up-regulated by CDCA), and sterol-27-hydroxylase, Intestinal Bile Acid Transporter, and Liver Bile Acid Transporter (these proteins are down regulated by CDCA).

20   The methods of the present invention are therefore useful in modulating the expression of these proteins as well.

The FXR ligands of the present invention may be FXR agonists, FXR antagonists, or FXR inverse agonists. By "agonist" is meant that the ligand stimulates a ligand-dependent FXR activity above any baseline levels present in the

25   absence of ligand. By "FXR activity" is meant the ligand-dependent direct or indirect inhibition of LXRα activity. By "antagonist" is meant that the ligand binds to FXR, and functions as a competitive or non-competitive inhibitor of FXR agonist activity. By "inverse agonist" is meant that the ligand will bind to FXR

and cause the suppression of FXR activity to a level lower than seen in the absence of any FXR ligand.

Also contemplated by the present invention are methods of lowering cholesterol in a mammal, comprising treating the mammal with a pharmaceutically

5   acceptable composition comprising an FXR antagonist or FXR inverse agonist.

In another aspect the present invention pertains to methods of stimulating or inhibiting the activity of an FXR receptor of a mammal by treating such a mammal with a pharmaceutically
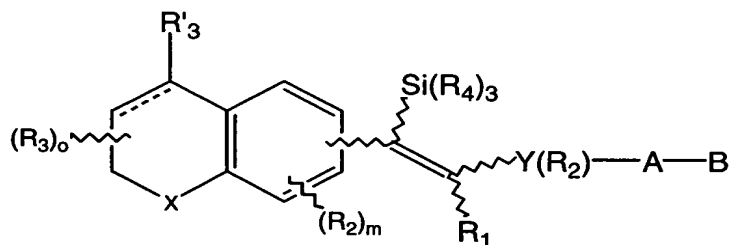
acceptable composition comprising a compound selected from the group consisting
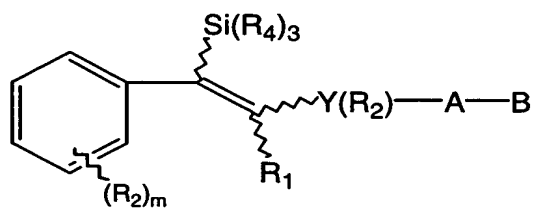of Formulas 1, 2, 3 and 4
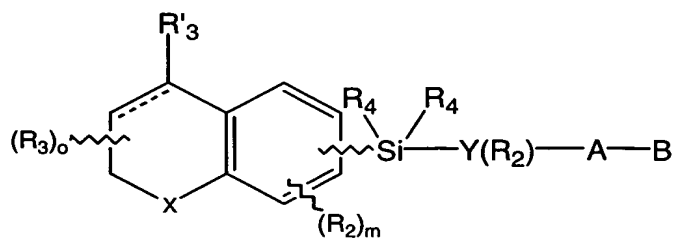
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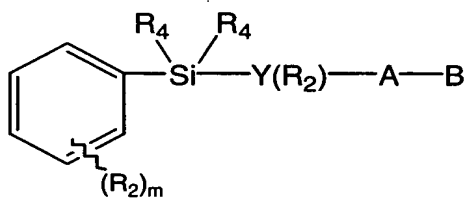
Formula 2

Formula 3

Formula 4

5

wherein the dashed line represents a bond or absence of a bond;

X is S, O, NR' where R' is H or alkyl of 1 to 6 carbons, or

X is $(C(R_1)_2)_n$ where R_1 is H or alkyl of 1 to 6 carbons, and n is an integer having the value of 0 or 1;

5   R_2 is hydrogen, lower alkyl of 1 to 6 carbons, F, Cl, Br, I, CF_3, fluoro substituted alkyl of 1 to 6 carbons, OH, SH, alkoxy of 1 to 12 carbons, or alkylthio of 1 to 12 carbons, benzyloxy or C_1 - C_{12} -alkylbenzyloxy;

R_3 is hydrogen, lower alkyl of 1 to 6 carbons or F;

m is an integer having the value of 0 - 3 in Formulas 1 and 3; and 0 - 5 in

10   Formulas 2 and 4;

o is an integer having the value of 0 - 4 when the dashed line represents absence of a bond, and 0 - 3 when the dashed line represents a bond;

R_3' is hydrogen, lower alkyl of 1 to 6 carbons, F or R_3' is hydrogen, lower alkyl of 1 to 6 carbons, F or $(R_{15})_r$-phenyl, $(R_{15})_r$-naphthyl, or $(R_{15})_r$- heteroaryl

15   where the heteroaryl group has 1 to 3 heteroatoms selected from the group consisting of O, S and N, r is an integer having the values of 0 - 5;

R_4 is alkyl of 1 to 8 carbons, or phenyl;

Y is a phenyl or naphthyl group, or heteroaryl selected from a group consisting of pyridyl, thienyl, furyl, pyridazinyl, pyrimidinyl, pyrazinyl, thiazolyl,

20   oxazolyl, imidazolyl and pyrrazolyl, said phenyl and heteroaryl groups being optionally substituted with one or two R_2 groups;

R_{15} is independently H, F, Cl, Br, I, NO_2, $N(R_8)_2$, $NH(R_8)$, COR_8, $NR_8CON(R_8)_2$, OH, $OCOR_8$, OR_8, CN, an alkyl group having 1 to 10 carbons, fluoro substituted alkyl group having 1 to 10 carbons, an alkenyl group having 1 to

25   10 carbons and 1 to 3 double bonds, alkynyl group having 1 to 10 carbons and 1 to 3 triple bonds, or a trialkylsilyl or trialkylsilyloxy group where the alkyl groups independently have 1 to 6 carbons;

11

A is $(CH_2)_q$ where q is 0-5, lower branched chain alkyl having 3-6 carbons, cycloalkyl having 3-6 carbons, alkenyl having 2-6 carbons and 1 or 2 double bonds, alkynyl having 2-6 carbons and 1 or 2 triple bonds;

B is hydrogen, COOH, NO_2, $P(O)(OH)_2$, $P(O)(OH)OR_8$, $P(O)(OR_8)_2$,

5   SO_2OH, $SO_2(OR_8)$, $COOR_8$, $CONR_9R_{10}$, $-CH_2OH$, CH_2OR_{11}, CH_2OCOR_{11}, CHO, $CH(OR_{12})_2$, $CHOR_{13}O$, $-COR_7$, $CR_7(OR_{12})_2$, $CR_7OR_{13}O$, or tri-lower alkylsilyl, where R_7 is an alkyl, cycloalkyl or alkenyl group containing 1 to 5 carbons, R_8 is an alkyl group of 1 to 10 carbons or trimethylsilylalkyl where the alkyl group has 1 to 10 carbons, or a cycloalkyl group of 5 to 10 carbons, or R_8 is phenyl or lower

10   alkylphenyl, R_9 and R_{10} independently are hydrogen, an alkyl group of 1 to 10 carbons, or a cycloalkyl group of 5-10 carbons, or phenyl or lower alkylphenyl, R_{11} is lower alkyl, phenyl or lower alkylphenyl, R_{12} is lower alkyl, and R_{13} is divalent alkyl radical of 2-5 carbons, or a pharmaceutically acceptable salt of said compound.

15   In a preferred embodiment of this latter aspect of the invention, a mammal suffering from hypercholesterolemia or hyperlipoproteinemia is treated with a pharmaceutically acceptable composition comprising an FXR antagonist selected from the group of such compounds. Preferably said mammal is a human.

In another preferred embodiment of the same aspect of the invention, a

20   mammal suffering from hypocholesterolemia is treated with a pharmaceutically acceptable composition comprising an FXR agonist selected from the group of such compounds. Preferably said mammal is a human.

Other aspects and embodiments of the invention are contained in the disclosure that follows and the claims that conclude this specification.

25

Detailed Description of the Invention

The present invention is directed to methods for modulating the activity of a mammalian FXR receptor, preferably the human FXR protein.

Such methods involve the use of compounds which will bind the FXR receptor, thereby affecting the ability of FXR to exert its biological effects, either directly or by blocking the ability of a naturally occurring ligand to exert its affects. The FXR ligands of the present invention may be FXR antagonists, FXR agonists,

5   or FXR inverse agonists. Preferably, although not necessarily, the FXR ligands have substantially no activity at the retinoid nuclear receptors, RAR and RXR. In another embodiment, the FXR ligand may be a bi-specific compound able to bind and modulate both RXR and FXR.

Also included within the scope of the invention are aspects directed to

10   methods for lowering the plasma concentration of cholesterol in a mammal, comprising treating the mammal with a pharmaceutically acceptable composition comprising an FXR antagonist or FXR inverse agonist.

Also included are aspects of the invention directed to methods for increasing the plasma concentration of cholesterol in a mammal pathologically

15   deficient in cholesterol through the use of an FXR agonist.

Another aspect of the invention concerns methods for lowering the concentration of bile acids in a mammal, comprising the use of an FXR antagonist or inverse agonist in a pharmacologically acceptable carrier. Alternatively, in another aspect of the invention an FXR agonist is used to increase the synthesis of

20   bile acids in a patient deficient in bile acid synthesis.

While not wishing to be bound by theory, the Applicants believe that the FXR receptor, when bound by an FXR agonist, functions to inhibit the transcription of the oxysterol receptor LXRα, which in turn activates transcription of Cyp7a. Repression of transcription of this key enzyme in the biosynthesis of bile acids

25   therefore results in a lower concentration of bile acids within the body; high bile acid concentrations have been associated with a heightened risk of colon cancer.

As an aid in the further understanding of this invention, Applicants offer the following Examples, which are intended to illustrate the invention, but not to limit the scope of the claims.

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Materials and Methods

    All mammalian expression vectors were derivatives of the

bacterial/mammalian shuttle vector pCMX, an expression vector containing the

5   cytomegalovirus (CMV) promoter/enhancer, followed by a bacteriophage T7

promoter for transcription of the cloned gene in vitro. Plasmid pCMX also contains

the SV40 small t intron/poly adenylation signal sequence, polyoma virus

enhancer/origin and the SV40 enhancer/origin of plasmid CDM8 (*see* Seed, *Nature*

329:840-842 (1987), hereby incorporated by reference herein) cloned into the large

10 Pvu II fragment of pUC19. PUC19 is a commonly used cloning vector available

from New England Biolabs, Inc. This Pvu II fragment contains a Col E1 origin of

replication and an ampicillin resistence gene for plasmid selection, but lacks the

pUC19 polylinker cloning site. To create a new polylinker site, a synthetic

polylinker comprising the following restriction sites: 5'-KpnI, EcoRV, BamHI,

15 MscI, NheI-3' followed by a translational termination sequence inserted in all three

reading frames. *See* Umesono et al., *Cell* 65:1255-1266 (1991), hereby

incorporated by reference herein.

    The nucleic acid regions encoding the following full-length proteins were

cloned into pCMX. The sequences of these genes and/or their corresponding

20 polypeptides have the indicated GenBank accession numbers: rat FXR (accession

number U 18374); mouse FXR (accession number U 09416); human FXR

(accession number NM 005123); and human RXRα (accession number X 52773).

The GenBank information corresponding to these accession numbers is hereby

incorporated by reference herein in its entirety. The rat FXR amino acid sequence,

25 mouse FXR amino acid sequence, and human FXR amino acid sequence are

provided herein as SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3,

respectively. The human RXRα amino acid sequence is provided herein as SEQ

ID NO: 4.

GAL4 fusion proteins were constructed using standard molecular biological methods (see e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual* (2d ed. Cold Spring Harbor Laboratory Press 1989), incorporated by reference herein in its entirety) by inserting a nucleotide sequence encoding the indicated polypeptide

5   immediately downstream of the yeast GAL4 DNA-binding domain in plasmid pSG424, described in Sadowski et al., *Nucleic Acids Research* 17:7539, hereby incorporated by reference herein. The amino acid sequence of the yeast GAL4 DBD, hereby designated SEQ ID NO: 5, is as follows:

10   NH2-MKLLSSIEQA CDICRLKKLK CSKEKPKCAK CLKNNWECRY SPKTKRSPLT
     RAHLTEVESR LERLEQLFLL IFPREDLDM ILKMDSLQD IKALLTGLF VQDNVNKDAV
     TDRLASVETD MPLTLRQHRI SATSSSEESS NKGQRQLTVS-COOH

     Fusion proteins were made, as indicated above, using common molecular

15   biological techniques by creation of open nucleic acid reading frames encoding the indicated polypeptides, and cloning into the polylinker portion of pCMX.

     For GAL-L-RXR, the plasmid nucleic acids encoded amino acids Glu_{203} to Thr_{462} of human RXRα (SEQ ID NO: 4) fused to the GAL4 sequences. The junction between the carboxyl terminal section of GAL4 and the amino terminal

20   portion of the RXR LBD had the following structure:


```
                          EcoRI     Asp718   Sal/Xho
             GTA-TCG-CCG-GAA-TTC-GGT-ACC-GTC-GAG-GCC-GTG-CAG-GAG-..
             Val-Ser                          Glu-Ala-Val-Gln-Glu-..
25              GAL4 ->                         203 --> hRXRa LBD
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     This junction nucleotide sequence

          5'GTATCGCCGGAATTCGGTACCGTCGAGGCCGTGCAGGAG3'

30   is hereby designated SEQ ID NO: 6.

     For GAL-L-FXR, the plasmid nucleic acids encoded amino acids Leu_{181} to Gln_{469} of rat FXR (SEQ ID NO: 1) fused to the GAL4 sequences. The junction

between the carboxyl terminal section of GAL4 and the amino terminal portion of
the FXR LBD had the following structure:

```
        EcoRI  former
  5              |  KpnI/NaeI
        GTATCGCCGGAATTCGGGCTAAGGAAGTGCAGAGAGATGGGAATGTTGGCTGAATG
        ValSerProGluPheGlyLeuArgLysCysArgGluMetGlyMetLeuAlaGlu
        GAL4>|                 |<---rFXRa AA 181              |<---LBD
```

10   This junction nucleotide sequence (from 5' to 3')

GTATCGCCGGAATTCGGGCTAAGGAAGTGCAGAGAGATGGGAATGTTGGC

TGAATG

is hereby designated SEQ ID NO: 7.

RXR ligand binding domain (LBD) expression construct L-RXR contains

15   nucleotide residues encoding the SV40 Tag nuclear localization signal sequence

(from amino to carboxy ends:

APKKKRKVG (SEQ ID NO: 8)

located immediately upstream (i.e., to the 5' side on the coding strand) of a

20   nucleotide sequence encoding the human RXRα LBD (Glu$_{203}$ to Thr$_{462}$). CMX-

βgal contains the E. coli β-galactosidase coding sequence derived from plasmid

pCH110 (accession number U 02445) inserted downstream of the CMV promoter

in plasmid pCMX. RXRm contains a single point mutation changing Asp-322 to

Pro in the LBD of human RXRα.

25   Luciferase reporter plasmids (termed TK-Luc) were constructed by placing

the cDNA encoding firely luciferase immediately downstream from the herpes

virus thymidine kinase promoter (located at nucleotide residues −105 to + 51) of

the thymidine kinase nucleotide sequence), which is linked in turn to the various

response elements. The promoter region of the TK-Luc plasmids has the following

30   structure:

16

```
                                                                    SalI
                                   HindIII              SphI  PstI   |
                                      |                   |    | |
                    GGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCAAGCTTGCATGCCTGCAGG
  5            61   ---------+---------+---------+---------+---------+---------+  120


                                                                  BsiCI
                                                                  BstBI
 10                 XbaI BamHI                              EcoRI    |
                      |    |                                  |     | |
                            pUC18 _|_  HSV-TK
                    TCGACTCTAGAGGATCCGGCCCCGCCCAGCGTCTTGTCATTGGCGAATTCGAACACGCAG
              121   ---------+---------+---------+---------+---------+---------+  180
 15

                                       AvaII
                                AvaII*   |
 20                             AvaII |  |                   MluI
                                  | |  |                      |
                    ATGCAGTCGGGGCGGCGCGGTCCCAGGTCCACTTCGCATATTAAGGTGACGCGTGTGGCC
              181   ---------+---------+---------+---------+---------+---------+  240


 25                            PstI                           BglII
                                |                               |
                    |-- transcription start _          HSV-TK _|
                    TCGAACACCGAGCGACCCTGCAGCGACCCGCTTAACAGCGTCAACAGCGTGCCGCAGATC
              241   ---------+---------+---------+---------+---------+---------+  300
 30

                                                                  EheI
                    PaeR7I                                        NarI|
                      XhoI                                        KasI||
 35                    |                                          |||
                    TCTCGAGTCCGGTACTGTTGGTAAAATGGAAGACGCCAAAAACATAAAGAAAGGCCCGGC
              301   ---------+---------+---------+---------+---------+---------+  360


 40                                   MetGluAspAlaLysAsnIleLysLysGlyProAla  -
                                      |_ luciferase
                         XbaI                FokI
                           |                  |
                    GCCATTCTATCCTCTAGAGGATGGAACCGCTGGAGAGCAACTGCATAAGGCTATGAAGAG
 45           361   ---------+---------+---------+---------+---------+---------+  420
                    ProPheTyrProLeuGluAspGlyThrAlaGlyGluGlnLeuHisLysAlaMetLysArg
```

This nucleotide sequence (continuous from 5' to 3')

```
           5'-GGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCAAGCTTGCATGCCTGCAGG
             TCGACTCTAGAGGATCCGGCCCCGCCCAGCGTCTTGTCATTGGCGAATTCGAACACGCAG
             ATGCAGTCGGGGCGGCGCGGTCCCAGGTCCACTTCGCATATTAAGGTGACGCGTGTGGCC
             TCGAACACCGAGCGACCCTGCAGCGACCCGCTTAACAGCGTCAACAGCGTGCCGCAGATC
             TCTCGAGTCCGGTACTGTTGGTAAAATGGAAGACGCCAAAAACATAAAGAAAGGCCCGGC
             GCCATTCTATCCTCTAGAGGATGGAACCGCTGGAGAGCAACTGCATAAGGCTATGAAGAG-3'
```

is designated SEQ ID NO: 9.

Response elements were inserted in plasmid TK-Luc at the unique Hind III
site. The yeast GAL4 UAS$_G$ response element has the nucleotide sequence, and
was inserted in 4 direct repeats:

5   5'-CGACGGAGTACTGTCCTCCGAGCT-3' (SEQ ID NO: 10)

The hsp EcRE (ecdysone response element) was inserted into the Hind III
site of plasmid TK-Luc as six direct repeats of the following sequence:

5'-TGGACAAGTGCATTGAACCCTT-3' (SEQ ID NO: 11)

10

Example 1

Because FXR is known to bind to its response element as a heterodimer
with RXR, and because the heterodimer can be activated by ligands to RXR, a
15   mutant RXRα protein (RXR$_m$; also referred to as D322P in the Figures) was
constructed containing a single point mutation (Asp$_{322}$ to Pro) in the ligand binding
domain of RXR. The use of FXR-RXR$_m$ heterodimers, permits unambiguous
identification of modulators of FXR activity amongst test compounds.

Thus, a reporter plasmid was constructed containing 4 copies of the GAL4
20   response element UAS$_G$ positioned upstream of the firefly luciferase gene, which in
turn was under the control of the herpes simplex virus thymidine kinase (TK)
promoter. This reporter plasmid was cotransfected into African green monkey CV-
1 cells with an expression vector (pCMX, which contains the cytomegalovirus
CMV promoter located upstream of the cloning site) encoding either GAL-L-RXR
25   (comprising the LBD of RXRα from Glu$_{203}$ to Thr$_{462}$) and the DNA-binding
portion (amino acids 1-147)), of the yeast GAL4 gene product, or GAL-L-RXRm
(identical to GA-L-RXR but for the single Asp$_{322}$ → Pro point mutation in the LBD
of the RXR coding sequence).

18

Transient transfection of the CV-1 cells was performed as follows. CV-1 cells were cultured in Dulbecco's Modified Eagle's medium containing 10% resin-charcoal stripped fetal bovine serum (FBS), 50 units/ml penicillin G and 50 μg/ml streptomycin sulfate (termed DMEM-FBS). The day prior to transfection the cells

5   were plated to 50%-80% confluence.

Cells were transiently transformed by lipofection as described in Forman et al., *Cell* 83:803-12 (1995), hereby incorporated by reference herein. Liposomes (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-ammonium methyl sulfate, sold by Boehringer Mannheim under the name DOTAP) were formed according to the

10   manufacturer's instructions. The liposomes contained reporter gene constructs (300ng/10^5 cells), and either the GAL-L-RXR or GAL-L-RXR_M expression vector (20-50ng/10^5 cells). The cells and liposomes were incubated together for 2 hours. Liposomes were removed by aspiration, and the cells were then incubated for approximately 44 hours (± 2 hours) in the presence of various concentrations of the

15   RXR agonist LG 268 dissolved in dimethylsulfoxide (DMSO). The structure of LG 268 is as follows:
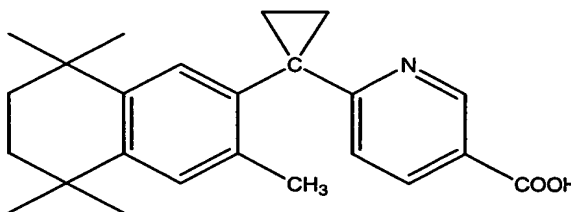
Following exposure to this compound, the cells were harvested and assayed

20   for the presence of luciferase activity. Cells were lysed in 0.1 M KPO4 (pH 7.8), 1.0% TRITON® X-100, 1.0 mM dithiolthreitol (DTT) and 2 mM ethylenediamine tetracetic acid (EDTA). Luciferase activity was measured by reaction of the cell lysates with luciferin in a reaction buffer comprising: 20 mM tricine, 1.07 mM Mg(CO_3)$_4$-Mg(OH)$_2$-5 $H_2$0, 2.67 mM $MgSO_4$-$7H_2$0, 0.1 mM EDTA, 0.5 mM

25   Sodium luciferin, 0.15 mg/ml Coenzyme A, 5 mM DTT, and 0.5 mM adenosine

triphosphate (ATP). Resulting chemiluminescence was measured in a luminometer. See de Wet et al., *Mol.Cell Biol.* 7:725 (1987) (hereby incorporated by reference herein).

5   All compounds were assayed in triplicate. Each experiment was repeated three or more times.

        Results are shown in Figure 1. As indicated therein, introduction of the RXR $Asp_{322} \rightarrow$ Pro mutation significantly decreases ($\geq$ 50-fold) the ligand-dependent transactivation activity of RXR_m in the presence of a known RXR agonist, LG268, compared to the transactivation ability of the unmutated ligand

10   binding domain of GAL-L-RXR.

Example 2

        Because the FXR transctivation activity requires formation of a heterodimer

15   with RXR, the following transactivation experiment was done to show that RXR_m retains its ability to potentiate FXR activity despite a having a non-functional ligand binding domain in an assay for ligand-dependent FXR activity.

        Full-length recombinant rat FXR and human RXR (or RXR_m) cloned into the cloning site of plasmid pCMX were cotransfected into CV-1 cells using the

20   transient transfection method substantially as described above. Additionally, reporter plasmids were cotransfected with the expression plasmids as described above.

        Luciferase reporter plasmids TK-Luc (described in Heyman et al., *Cell* 68:397 (1992), hereby incorporated by reference herein) were constructed by

25   placing the firefly luciferase cDNA coding sequence in frame immediately downstream from the herpes virus thymidine kinase promoter (located at nucleotide residues −105 to + 51) of the thymidine kinase nucleotide sequence). Six repeats of the EcRE response element, to which the DNA binding domain (DBD) of FXR

binds, was also included upstream of the reporter gene. Further details are
provided above.

Following transfection, the cells were incubated for approximately 44 hours
in either bovine bile extract, 5-10 μM of the FXR test compound AGN 10 (also
5   termed AGN 192337), or 100 nM of the RXR agonist LG 268, each dissolved in
DMSO. AGN 10 has the structure:
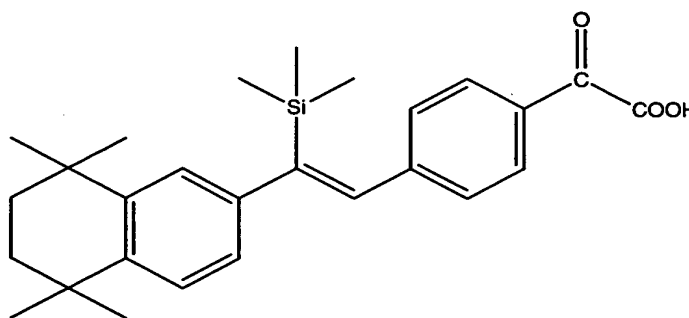
10   Bovine bile extract was obtained commercially from Sigma Chemicals, Inc.
One gram of the bile extract was dissolved in 50 ml water and adjusted to pH 4.0.
Material that was insoluble in water was extracted in 200 ml of methanol. Each of
these extracts was completely dried *in vacuo* at 40°C , then redissolved.

The cells were then lysed as described above, and luciferase activity was
15   measured as an indication of the extent of heterodimer-induced FXR
transactivation activity as described above Results are shown in Figure 2.

As can be seen, bovine bile extract has significant ability to potentiate FXR-
mediated transactivation in both FXR-RXR and FXR-RXR$_m$ cotransfected cells.
Also, the FXR ligand candidate AGN 10 has almost as much activity towards an
20   FXR target (in both FXR-RXR and FXR-RXR$_m$ assays). LG 268, shown in
Example 1 to have little FXR-specific activity, is able to cause transactivation of
the reporter gene when exposed to a heterodimer comprising rat FXR and RXR.

By contrast, the RXR ligand LG 268 has notable activity in the FXR-RXR-transfected cells, but not in the FXR-RXRm transfected cells.

These data therefore demonstrate that FXR transactivation potentiated by FXR ligands can be distinguished from transactivation caused by RXR-specific
5   ligand interaction with the RXR half of the active FXR-RXR heterodimer through the use of a mutated form of RXR. This Example, together with the data from Example 1, also suggests that RXRm is able to form a transactivationally active heterodimer with FXR even though RXRm is unable to bind ligand effectively. Similar results are seen using the full length human FXR protein.
10

Example 3:

The FXR-RXRm cotransfection methods employed in Example 2 were used to compare the FXR agonist activity of AGN 10 with the FXR activity of various
15   bile acids, including deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) which are known to be naturally-occurring FXR ligands. *See e.g.,* Wang et al., *Molec. Cell* 3:543-553 (May, 1999), hereby incorporated herein by reference.

CV-1 cells were cotransfected with mammalian expression vectors encoding full length FXR and RXRm, as well as with the luciferase reporter
20   plasmid used in the last experiment. The cells were then incubated with one of the following agents for approximately 44 hours at a concentration of 100 nM, unless otherwise indicated: LG 268; AGN 10 (10 µM); 7-ketolithocholic acid, 3,7-diketolithocholic acid; ursodeoxycholic acid; a-muricholic acid; murocholic acid; dehydrocholic acid; taurocholic acid; cholic acid (CA); lithocholic acid (LCA);
25   taurodeoxycholic acid; deoxycholic acid (DCA); glycochenodeoxycholic acid; taurochenodeoxycholic acid; and chenodeoxycholic acid (CDCA). All compounds were dissolved in DMSO, except glycochenodeoxycholic acid, taurochenodeoxycholic acid, taurodeoxycholic acid and taurocholic acid, which were dissolved in phosphate buffered saline (PBS). Following exposure to the

compounds, the cells were washed, lysed, and the luciferase activity measured as indicated above.

Figure 3 indicates that, of the tested compounds, AGN 10, 7-ketolithocholic acid, 3-7-diketolithocholic acid, lithocholic acid, deoxycholic acid, and

5   chenodeoxycholic acid have measurable FXR transactivating activity in this assay system. Of the naturally occurring bile acids only chenodeoxycholic acid (delivered at a concentration of 100 μM) showed a level of activity as great as that displayed by 10 μM AGN 10.

10   Example 4

A similar experiment was performed in order to determine the FXR transactivating activity of selected compounds as a function of ligand concentration. Transient cotransfection of full length rat FXR, human RXR and the

15   luciferase reporter plasmid was performed as above. A set of cotransfectant CV-1 cells was incubated in 1, 2, 10, and 20 μM AGN 10. Separate sets of cotransformants were given 1, 10, 20, 100, and 200 μM of either CDCA, DCA or LCA. All compounds were dissolved in DMSO. All transformant cells were permitted to incubate for approximately 44 hours with the indicated compound,

20   then the amount of transactivation activity was measured using the luciferase assay, as indicated above.

Figure 4 shows the results of this experiment. As can be seen, the concentration-dependent ability of AGN 10 to agonize FXR transactivation activity rises in a dose-dependent fashion between concentrations of 1 and 10 μM. At this

25   latter concentration, the FXR agonist activity of AGN 10 appears to be at a maximum in this assay system.

By contrast, as Figure 4 shows, the activities of CDCA and DCA remain at baseline up to 10 μM, then rise in approximately linear fashion to their maxima, at about 100 μM. LCA also has a very low activity until reaching a concentration of

10 µM, but then the activity rises between 10 and 100 µM. The maximum activity

of DCA and LCA is respectively about ½ and ¼ that of AGN 10, while the

maximum activity of CDCA at 100 µM is roughly that of AGN 10 at 10 µM.

5   Example 5

        To screen additional compounds for FXR agonist activity, a panel of

compounds was assembled. These compounds were: TTNPB (which has FXR

activity), Am 580 (which does not have FXR activity), juvenile hormone (JH) III

10   (50 µM) (an FXR agonist); all-trans retinoic acid (a naturally occurring RAR

agonist); 9-cis retinoic acid (an RXR agonist); LG 268, LG 69 (synthetic RXR

agonists); and 28 compounds designated AGN 1 through AGN 28.

        The structures of AGN 1 through AGN 28 are provided in Figure 5a, 5b, 5c,

and 5d.

15   The structure of Am 580 is:
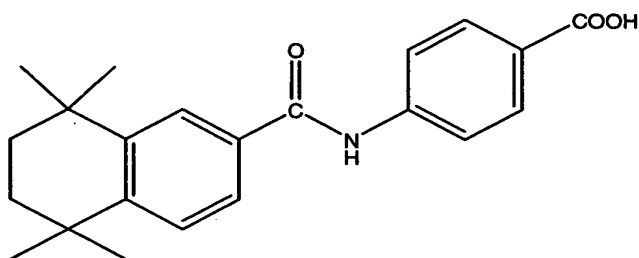
The structure of LG 69 is:

20

24
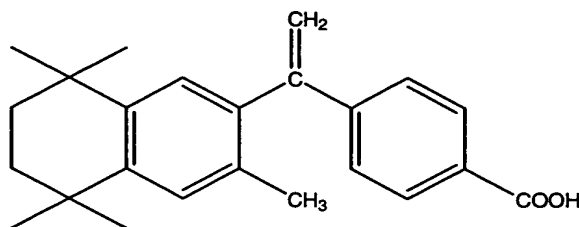
The structure of juvenile hormone III (JH III) is:

5
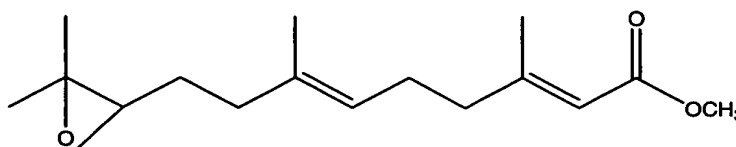
The structure of TTNPB is:

10
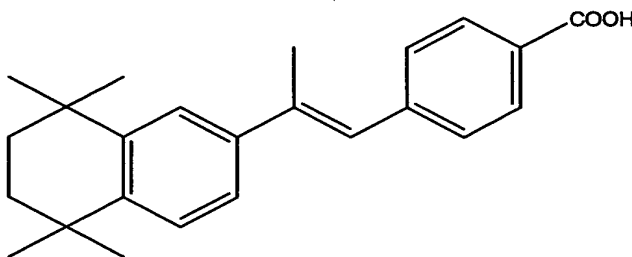
These compounds were assayed for FXR modulating activity using the transactivation assay described above. All compounds were dissolved in DMSO. As above, the reporter plasmid contained 6 copies of the ecdysone response element (EcRE) placed along with the herpes virus thymidine kinase promoter
15 upstream of the firefly luciferase gene. The reporter plasmid was transfected into CV-1 cells, either a) alone, b) with an expression plasmid encoding full length FXR, c) with an expression plasmid encoding full length RXRα, d) with two expression plasmids encoding full length FXR and RXRα, respectively, and e) with two expression plasmids encoding full length FXR and RXR_m, respectively.
20 Plasmid constructions, transfection, incubation with test compounds, and luciferase activity assays were performed as described in Example 1.

The results are shown in Figure 6. Virtually no transactivation activity was seen in the absence of FXR and RXRα. Upon transfection of the cells with FXR alone, no significant increase in the baseline activity was seen. When cells were transfected with RXRα alone, no increase in activity over baseline could be seen.

5   Cells cotransfected with the reporter plasmid and both FXR and RXRα expression plasmids generally responded with a considerable increase in luciferase activity upon challenge with the test compounds. Those cells given TTNPB, AGN 10 and AGN 20 again gave the highest activity response. Finally, cells cotransfected with both FXR and RXR_m provided a much more discriminating activity profile; the

10   overall extent of transactivation activity was universally decreased, and only TTNPB, AGN 10 and AGN 20 showed significant levels of transctivation activity.

However, upon cotransfection with FXR and either RXRα or RXRm, significant ligand-dependent transactivation occurs. This is almost certainly due to the formation of FXR-RXR heterodimers, and shows that the ability of both FXR

15   and RXR to promote transactivation is highly dependent upon heterodimer formation. Use of RXRm as a heterodimeric partner with FXR permits FXR-specific ligands to be distinguished from those acting on the RXR half of the heterodimer pair.

Finally, the data show that AGN 10 and AGN 20 are FXR agonists, and that

20   ligand-dependent activation of FXR can occur without ligand-dependent priming of RXR.

The following example provides a detailed description of compounds having FXR modulating activity, as well as methods of making such compounds. Those of skill in the art will recognize that the structures of the FXR agonists AGN

25   10, CDCA and DCA may be used to select one or more common feature for the molecular modeling of other FXR agonists. Without limitation, some of these features include the presence of an acidic group on the right hand side of the structure, the presence of a ring or ringlike structure in the position of the

trimethylsilane group of AGN 10, and perhaps the addition of one or more hydroxyl group to the psuedo naphthyl nucleus of these compounds.

Similarly, much is known about the type of modifications that may be made to an agonist to convert it into an antagonist. Thus, creation of FXR antagonists

5   or inverse agonists, given the structure of a strong receptor agonist like AGN 10, is possible. Indeed, such modifications to a receptor agonist have already been made in the design of antagonists and inverse agonists of the retinoid receptors. See e.g., U.S. Patent 5,776,699, incorporated by reference herein. Since an agonist binds to the LBD of the nuclear hormone to exert its effect, the modification of such an

10   agonist to create a receptor antagonist generally involves retention of the same general structure as the agonist (thus permitting the antagonist to continue to bind the receptor) combined with the addition of somewhat "bulky" groups to prevent the specific conformational changes of the receptor that result in activation of the gene transcriptional functions of the receptor.

15   Thus, in the present case, an FXR antagonist or inverse agonist would be expected by the person or ordinary skill in the art to have a structure similar to that of AGN 10, but to contain modifications including, without limitation, addition of an aryl group to the six-membered non-aromatic ring, particularly at the uppermost position of the ring (relative to Formulae 1-5, infra); addition of an alkyl group

20   greater than C2, or an aryl group at the silyl moiety, and addition of an aryl group to the unsubstituted carbon of the double bond to the right of the trimethylsilyl substitution of AGN 10. Other such modifications will be apparent to the person of skill in the art, and are contained in the following Example and the claims that conclude this specification.

25

Example 6: Preferred FXR-Modulating Compounds, and their Synthesis

GENERAL EMBODIMENTS AND SYNTHETIC METHODOLOGY
Definitions

5   The term alkyl refers to and covers any and all groups which are known as
normal alkyl, branched-chain alkyl and cycloalkyl. The term alkenyl refers to and
covers normal alkenyl, branch chain alkenyl and cycloalkenyl groups having one or
more sites of unsaturation. Similarly, the term alkynyl refers to and covers normal
alkynyl, and branch chain alkynyl groups having one or more triple bonds.

10   Unless specified otherwise, lower alkyl means the above-defined broad
definition of alkyl groups having 1 to 6 carbons in case of normal lower alkyl, and
as applicable 3 to 6 carbons for lower branch chained and cycloalkyl groups.
Lower alkenyl is defined similarly having 2 to 6
carbons for normal lower alkenyl groups, and 3 to 6 carbons for branch chained and

15   cyclo- lower alkenyl groups. Lower alkynyl is also defined similarly, having 2 to 6
carbons for normal lower alkynyl groups, and 4 to 6 carbons for branch chained
lower alkynyl groups.

          The term "ester" as used here refers to and covers any compound falling
within the definition of that term as classically used in organic chemistry. It

20   includes organic and inorganic esters. Where **B** of **Formula 1, 2, 3** or **4** is -COOH,
this term covers the products derived from treatment of this function with alcohols
or thiols preferably with aliphatic alcohols having 1-6 carbons. Where the ester is
derived from compounds where **B** is -CH$_2$OH, this term covers compounds derived
from organic acids capable of forming esters including phosphorous based and

25   sulfur based acids, or compounds of the formula -CH$_2$OCOR$_{11}$ where **R$_{11}$** is any
substituted or unsubstituted aliphatic, aromatic, heteroaromatic or aliphatic
aromatic group, preferably with 1-6 carbons in the aliphatic portions.

By "synthetic compound" is meant an organic compound that does normally not occur in a mammal. Specifically, a synthetic compound is meant to exclude a naturally occurring bile acid.

By "ligand" is meant a compound able to bind to a given biological
5   molecule, or a set of isotypes of a given biological molecule, with a high degree of avidity and specificity.

By "synthetic FXR ligand" is meant a synthetic compound that is able to bind to an FXR receptor protein with a high degree of avidity and specificity.

Unless stated otherwise in this application, preferred esters are derived from
10   the saturated aliphatic alcohols or acids of ten or fewer carbon atoms or the cyclic or saturated aliphatic cyclic alcohols and acids of 5 to 10 carbon atoms. Particularly preferred aliphatic esters are those derived from lower alkyl acids and alcohols. Also preferred are the phenyl or lower alkyl phenyl esters.

Amide has the meaning classically accorded that term in organic chemistry.
15   In this instance it includes the unsubstituted amides and all aliphatic and aromatic mono- and di- substituted amides. Unless stated otherwise in this application, preferred amides are the mono- and di-substituted amides derived from the saturated aliphatic radicals of ten or fewer carbon atoms or the cyclic or saturated aliphatic-cyclic radicals of 5 to 10 carbon atoms. Particularly preferred amides are
20   those derived from substituted and unsubstituted lower alkyl amines. Also preferred are mono- and disubstituted amides derived from the substituted and unsubstituted phenyl or lower alkylphenyl amines. Unsubstituted amides are also preferred.

Acetals and ketals include the radicals of the formula-CK where K is
25   $(-OR)_2$. Here, R is lower alkyl. Also, K may be $-OR_7O-$ where R_7 is lower alkyl of 2-5 carbon atoms, straight chain or branched.

A pharmaceutically acceptable salt may be prepared for any compound in this invention having a functionality capable of forming a salt, for example an acid functionality. A pharmaceutically acceptable salt is any salt which retains the

activity of the parent compound and does not impart any deleterious or untoward effect on the subject to which it is administered and in the context in which it is administered.

5   Pharmaceutically acceptable salts may be derived from organic or inorganic bases. The salt may be a mono or polyvalent ion. Of particular interest are the inorganic ions, sodium, potassium, calcium, and magnesium. Organic salts may be made with amines, particularly ammonium salts such as mono-, di- and trialkyl amines or ethanol amines. Salts may also be formed with caffeine, tromethamine and similar molecules. Where there is a nitrogen sufficiently basic as to be capable

10   of forming acid addition salts, such may be formed with any inorganic or organic acids or alkylating agent such as methyl iodide. Preferred salts are those formed with inorganic acids such as hydrochloric acid, sulfuric acid or phosphoric acid. Any of a number of simple organic acids such as mono-, di- or tri- acid may also be used.

15   Many compounds of the present invention have *trans* and *cis* (**E** and **Z**) isomers. Specific orientation of substituents relative to a double bond is indicated in the name of the respective compound, and/or by specifically showing in the structural formula the orientation of the substituents relative to the double bond. Unless it is specifically stated otherwise the invention covers *trans* as well as *cis*

20   isomers. Where the chemical name indicates a specific isomer, that designation by name is intended to control over a structure that may be ambigously drawn or shows a different isomer.

Some of the compounds of the present invention may contain one or more chiral centers and therefore may exist in enantiomeric and diastereomeric forms.

25   The scope of the present invention is intended to cover all isomers *per se*, as well as mixtures of *cis* and *trans* isomers, mixtures of diastereomers and racemic mixtures of enantiomers (optical isomers) as well.

With reference to the symbol **Y** in **Formulas 1, 2, 3** and **4** the preferred compounds of the invention are those where **Y** is phenyl, naphthyl, pyridyl, thienyl

or furyl. Even more preferred are compounds where **Y** is phenyl. As far as substititutions on the **Y** (phenyl) and **Y** (pyridyl) groups are concerned, compounds are preferred where the phenyl group is 1,4 (*para*) substituted and where the pyridine ring is 2,5 substituted. (Substitution in the 2-position in the "pyridine"

5   nomenclature corresponds to substitution in the 6-position in the "nicotinic acid" nomenclature.) In the presently preferred compounds of the invention there is no R_2 substituent on the **Y** group.

         The **A-B** group of the preferred compounds is $(CH_2)_q COOH$ or $(CH_2)_q$-$COOR_8$, where R_8 is defined as above. Even more preferably **q** is zero and

10   R_8 is lower alkyl or (trialkylsilyl)ethyl (or alkyl) or (trimethylsilyl)ethyl and more prefereably R_8 is hydrogen. Compounds are also preferred where the **A-B** group is CH_2OH.

         With reference to the group X in **Formulas 1** and **3**, in the presently preferred compounds of the invention **X** is O (chroman or chromene compounds)

15   or **X** represents $C(R_1)_2$ (tetrahydronaphthalene or dihydronaphthalene derivatives). Even more preferably R_1 of $C(R_1)_2$ is methyl.

         R_2 is preferably hydrogen or lower alkyl, even more preferably methyl and R_2 is preferably in the 3 position of the tetrahydronaphthalene and dihydronaphthalene moiety, and preferably in the 8 position of the chroman,

20   chromen, thiochroman, thiochromen, dihydro or tetrahydroquinoline moiety. When R_2 is other than hydrogen then preferably there is only one R_2 substituent in the aromatic portion of the condensed ring.

         R_3 is preferably hydrogen or methyl. Presently most preferred substitution of the non-aromatic portion of the condensed ring when the dashed line represents

25   absence of a bond in **Formulas 1** and **3** is such that there are geminal dimethyl groups in the 2 or 4 positions, or in both when **X** is a heteroatom, and geminal dimethyl groups in the 5 and 8 positions when the condensed ring is tetrahydronaphthylene and geminal dimethyl groups in the 5-position when the condensed ring is dihydronaphthalene. When the dashed line represents a bond, R_3

is preferably $(R_{15})_r$-phenyl, $(R_{15})_r$-naphthyl, or $(R_{15})_r$- heteroaryl, more preferably $(R_{15})_r$-phenyl, or $(R_{15})_r$-thienyl and R_{15} preferably is an alkyl group. As represented herein, numbering of the bicyclic ring structure is as follows.

5   In the presently preferred compounds of the invention the silicon containing substituent is preferably attached at the 6 position of the chroman, chromene, thiochroman, thiochromene, tetrahydroquinoline or dihydroquinoline nucleus, and to the 2 position of the tetrahydronaphthalene or dihydronaphthalene nucleus.

The present specific examples of the compounds of the invention are disclosed in **TABLE 1** with reference to **Formula 5** and **Formula 6** and their

10   preparation by the presently preferred synthetic methodology is described in the experimental section of this application.
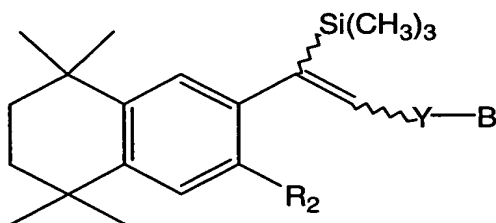
Formula 5
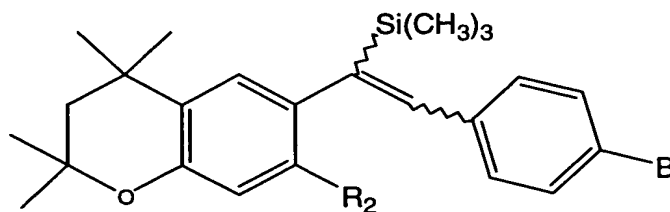
Formula 6

Table 1

Compound	Formula	R₂	Y	B
3	5	CH_3	1,4 substituted phenyl	CH_2OH
4	5	CH_3	1,4 substituted phenyl	COOEt
5	5	CH_3	1,4 substituted phenyl	COOH
6	5	H	1,4 substituted phenyl	CH_2OH
7	5	H	1,4 substituted phenyl	COOEt
8	5	H	1,4 substituted phenyl	COOH
10	6	H	-------	COOEt
11	6	H	-------	COOH
14	5	CH_3	2,5 substituted thienyl	CH_2OH
15	5	CH_3	2,5 substituted thienyl	COOEt
16	5	CH_3	2,5 substituted thienyl	COOH
17	5	H	2,5 substituted thienyl	CH_2OH
18	5	H	2,5 substituted thienyl	COOEt
19	5	H	2,5 substituted thienyl	COOH

The compounds of the invention can be made by the generalized synthetic route shown in **Reaction Scheme 1, 1a** and **Reaction Scheme 2.**

Referring now to **Reaction Scheme 1** and **Reaction Scheme 1a,** a presently
5   preferred synthetic route to compounds of the invention of **Formula 3** is disclosed.
In accordance with **Scheme 1** a bromoarylmethyl alcohol compound of **Formula 7**
is the starting material. In **Formula 7** the symbols Y and R_2 are defined as in
connection with **Formulas 1 - 4.** Examples for the compounds of **Formula 7**
which are used for the synthesis of presently preferred exemplary compounds of
10   the invention are 4-bromobenzyl alcohol and (5-bromothiophen-2-yl)-methyl
alcohol. Other examples are 3-bromobenzyl alcohol, (6-bromopyridin-3-yl)methyl
alcohol and (5-bromofuran-2-yl)methyl alcohol.   These starting materials are
either available commercially or can be readily obtained in accordance with the
chemical literature. The alcohols of **Formula 7** are reacted with a reagent that
15   introduces a protecting group on the primary alcohol function. An example of a
suitable reagent to introduce the protecting group and one that is used in the
synthesis of the presently preferred compounds of the invention is *tert-*
butyldiphenylsilyl chloride shown in **Reaction Scheme 1.** The product of the
reaction with *tert-*butyldiphenylsilyl chloride (conducted in the presence of base) is
20   a (bromoaryl)methyl *t-*butyldiphenylsilyl ether of **Formula 8.**

Br-Y(R$_2$)-CH$_2$OH   ⟶   Br-Y(R$_2$)-CH$_2$OSiPh$_2$tBu

Formula 7   Ph$_2$tBuSiCl, Et$_3$N, CH$_2$Cl$_2$ Formula 8

TMS-CCH
Pd(PPh$_3$)$_2$Cl$_2$
Et$_3$N, CuI

TMS-CC-Y(R$_2$)CH$_2$OSiPh$_2$tBu   (1) (cyclohexyl)$_2$BH

Formula 9   TMS=trimethylsilyl   (2)

Formula 10

(3) NaOH, H$_2$O$_2$

(4) Tetrabutylammonium fluoride

Formula 11

(1) MnO$_2$

(2) MnO$_2$, NaCN, EtOH

Formula 12   HOMOLOGS AND DERIVATIVES

REACTION SCHEME 1

TMS-CCH

$$\text{Br-Y(R}_2\text{)-A-B} \xrightarrow[\text{Et}_3\text{N}]{\text{Pd(PPh}_3\text{)}_2\text{Cl}_2} \text{TMS-CC-Y(R}_2\text{)-A-B}$$
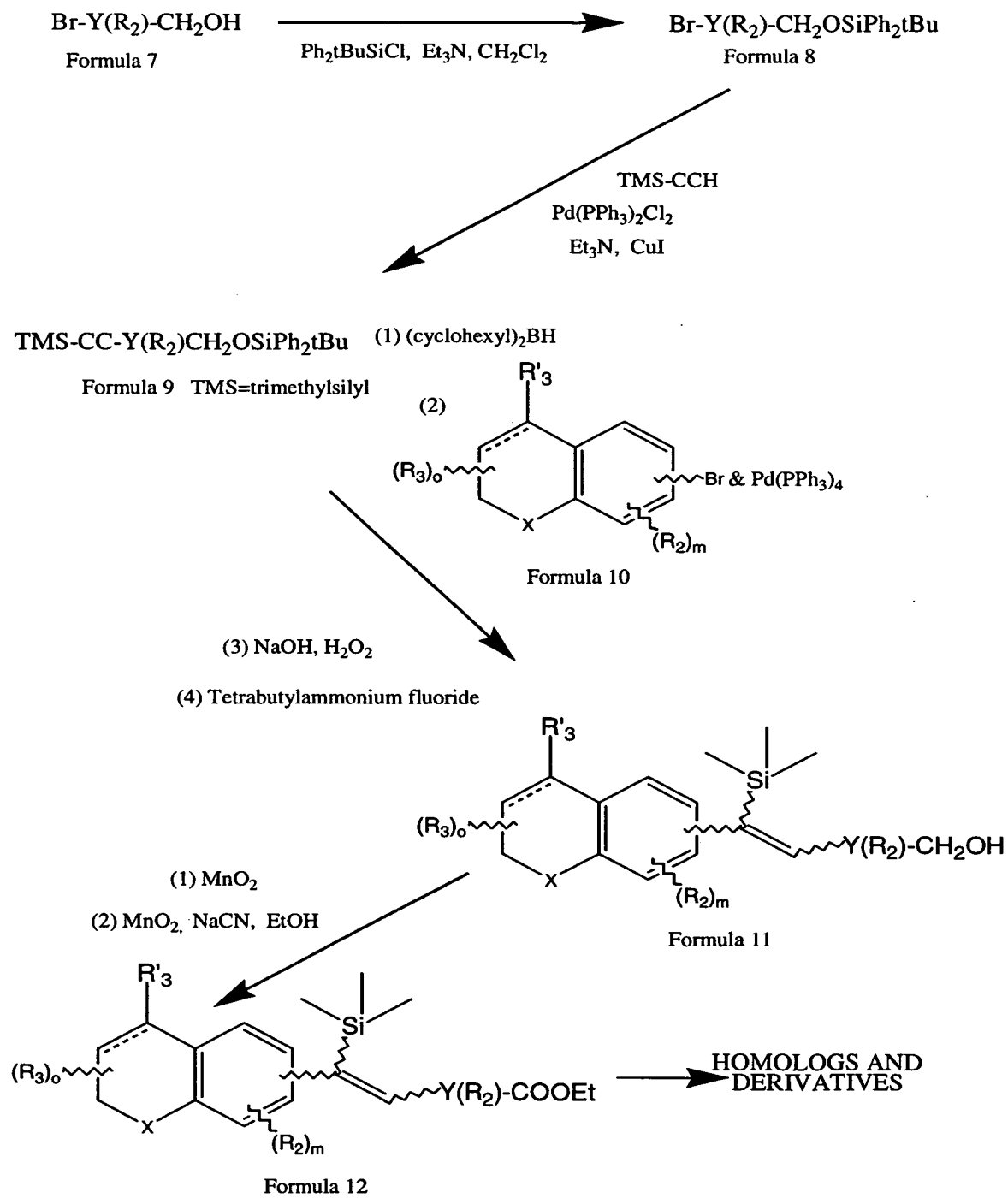
Formula 13   Formula 14
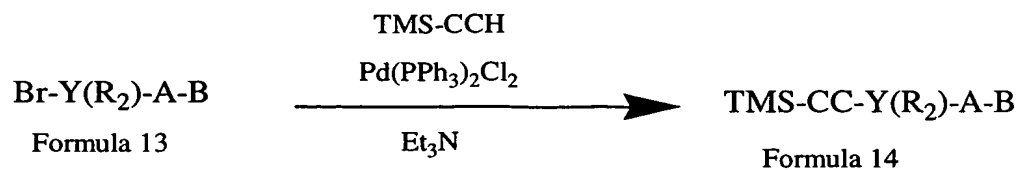
TMS-CC-Y(R$_2$)-A-B

Formula 14

(1) (cyclohexyl)$_2$BH
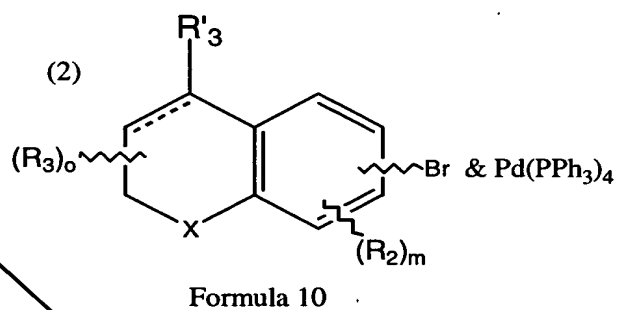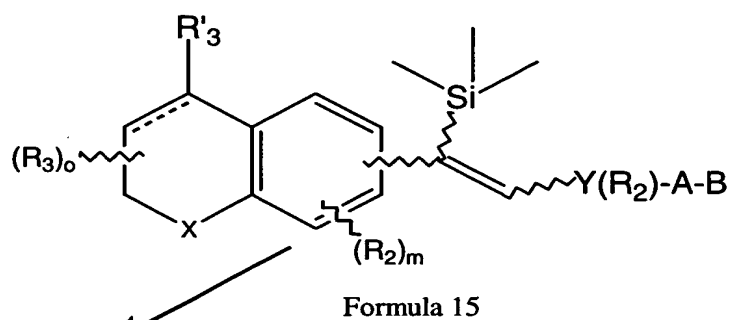
(2)

Formula 10

(3) NaOH, H$_2$O$_2$

Formula 15

HOMOLOGS AND DERIVATIVES

REACTION SCHEME 1a

The (bromoaryl)methyl *t*-butyldiphenylsilyl ether of **Formula 8** is reacted with (trimethylsilyl)acetylene in the presence of bis(triphenylphosphine)palladium (II) chloride catalyst, copper (I) iodide and a suitable base such as triethylamine. The latter coupling reaction of a bromoaryl compound with

5   (trimethylsilyl)acetylene in the presence of a palladium complex catalyst *per se* is well known in the art, and is described for example in United States Patent Nos. 5,663,347 and 5,808,083 the specification of which are expressly incorporated herein by reference. The product of the coupling reaction with (trimethylsilyl)acetylene is a ((trimethylsilyl)ethynylaryl)methyl *t*-

10   butyldiphenylsilyl ether of **Formula 9**.

Referring now to **Reaction Scheme 1a**, the starting material is a bromoaryl compound of **Formula 13** where the symbols **Y**, **R₂**, **A**, and **B** are again defined as in connection with **Formulas 1-4**. Examples for the starting compounds of **Formula 13** are ethyl 4-bromobenzoate, ethyl 6-bromonicotinate, ethyl 2-

15   bromothiophene-3-carboxylate and ethyl 2-bromofuran-3-carboxylate. These and analogous bromoaryl esters are readily available in accordance with the chemical literature. The bromoaryl compound of **Formula 13** is reacted with (trimethylsilyl)acetylene in the same manner as described in **Reaction Scheme 1**, to provide the (trimethylsilyl)ethynylaryl compounds of **Formula 14**. It will be, of

20   course, readily apparent to those skilled in the art that instead of the bromo derivatives the appropriate iodo derivatives can also be used in the the compounds of **Formula 7** and **Formula 13**.

In the next step of the reaction sequence shown both in **Reaction Scheme 1** and **1a**, the (trimethylsilyl)ethynylaryl compounds of **Formula 9 (Scheme 1)** or of

25   **Formula 14 (Scheme 1a)** is reacted with bis(cyclohexanyl)borane, which is prepared by reacting borane methyl sulfide with two equivalents of cyclohexene in an ethereal solvent such as tetrahydrofuran (THF). Bis(cyclohexanyl) borane, which is indicated in the reaction scheme, reacts with the (trimethylsilyl)ethynylaryl compounds of **Formula 9 (Scheme 1)** or of **Formula**

14 (**Scheme 1a**) to form an intermediate adduct.   This adduct is reacted in the

presence of tetrakis(triphenylphosphine)palladium (0) in an ethereal solvent, such

as THF, with a bromoaryl compound of **Formula 10**. The coupling of the bromo

(or iodo) aryl compound of **Formula 10** with the adduct is typically conducted

5   under reflux conditions in an inert (argon) gas atmosphere. Base (NaOH) and

hydrogen peroxide is then added to the reaction mixture to provide the

(trimethylsilyl)vinyl product of **Formula 15** in **Scheme 1a**. In accordance with

Scheme 1 product of the coupling reaction still includes the diphenyl-*t*-butylsilyl

protecting group which is removed by treatment with tetrabutylammonium fluoride

10   to give the (trimethylsilyl)vinyl) aryl methyl alcohol derivatives of **Formula 11**.

The condensed cyclic bromoaryl compounds of **Formula 10** which are used

in the coupling reaction are available in accordance with the chemical scientific or

patent literature, or can be obtained within the skill of the ordinary artisan in

analogy to synthetic processes known in the scientific or patent literature.

15   Examples for compounds of **Formula 10** which are used for the preparation of

presently preferred compounds of the invention are 2-bromo-3,5,5,8,8-

pentamethyl-5,6,7,8-tetrahydronaphthalene, 2-bromo-5,5,8,8-tetramethyl-5,6,7,8-

tetrahydronaphthalene and 6-bromo-2,2,4,4-tetramethylchroman. Further

examples are 6- or 7-bromo-4,4-dimethylchroman, 6- or 7-bromo-4,4-

20   dimethylthiochroman and 2 or 3 bromo tetrahydroquinoline derivatives which are

available in accordance with the teachings of United States Patent Nos. 5,348,972,

5,053,523 and 5,877,207 the specifications of which are incorporated herein by

and

reference. As still further examples United States Patent Nos. 5,278,318, ∧

5,407,937, ~~and 5,407,937~~ describe 2-alkyl and/or 4-alkyl substituted thiochromans

25   also substituted with a bromo group in the 6 position. United States Patent No.

5,346,895

∧ ~~5,346,585~~ describes 2-alkyl and/or 4-alkyl substituted thiochromans substituted

with a bromo group in the 7 position. United States Patent Nos. 5,324,744,

5,346, 895

5,348,975 and ~~5,346,585~~ describe 2-alkyl and/or 4-alkyl substituted chromans

∧

substituted with a bromo group in the 7 position. United States Patent No.

5,348,972 describes 4-alkyl substituted tetrahydroquinoline compounds substituted with a bromo group in the 2-position. The specifications of United States Patent Nos. 5,278,318, 5,324,744, 5,346,585, 5,348,975, and 5,407,937 are also expressly incorporated herein by reference.

5   Condensed cyclic bromoaryl compounds of **Formula 10** where the dashed line represents a bond, and particularly those where the dashed line represents a bond and the R'_3 substituent is an aryl or heteroaryl group, can be obtained from the corresponding brominated chroman-4-one, thiochroman-4-one, tetrahydroquinoline-4-one, and tetrahydronaphthalenone derivatives by first

10   forming the (trifluoromethyl)sulfonyloxy derivatives from the oxo functionality, and thereafter reacting those with an (organometallic) derivative that introduces the R'_3 group in analogy to the reactions described in United States Patent No. 5,877,207. Alternatively, the compounds of the invention where the dashed line represents a bond and the R^1_3 substituent is an aryl or heteroaryl group, can be

15   obtained from the corresponding (trimethylsilyl)vinyl derivatives that include an oxo function in the 4-position of the chroman, thiochroman or tetrahydroquinoline, and in the 8-position of tetrahydronaphthalene nucleus. These reactions are also conducted through the (trifluoromethyl)sulfonyloxy intermediates, in analogy to the teachings of United States Patent No. 5,877,207.

20   Referring now again to **Reaction Scheme 1**, the primary alcohol derivatives of **Formula 11** are compounds within the scope of the invention, particularly within the scope of **Formula 3**. The primary alcohols can be oxidized to the ester stage, for example as shown in **Scheme 1**, by treatment with manganese dioxide that first oxidizes the primary alcohol to the aldehyde stage, and thereafter by

25   treatment of the aldehyde with manganese dioxide and sodium cyanide in alcohol, to provide the ethyl ester derivatives of **Formula 12.** The compounds of **Formula 11**, and **12** in **Reaction Scheme 1**, and the compounds of **Formula 15** in **Reaction Scheme 1a** can be converted to further compounds of the invention by synthetic procedures which are well known in the art. This is indicated in **Reaction**

Schemes 1 and **1a** as conversion to "Homologs and Derivatives" and the transformations symbolized here primarily refer to reactions of the group designated **A-B** in the formulas.   In these and related reactions the following well known and published general principles and synthetic methodology can be

5   employed.

Carboxylic acids are typically esterified by refluxing the acid in a solution of the appropriate alcohol in the presence of an acid catalyst such as hydrogen chloride or thionyl chloride. Alternatively, the carboxylic acid can be condensed with the appropriate alcohol in the presence of dicyclohexylcarbodiimide (DCC)

10   and 4-(dimethylamino)pyridine (DMAP). The ester is recovered and purified by conventional means. Acetals and ketals are readily made by the method described in March, "Advanced Organic Chemistry," 2nd Edition, McGraw-Hill Book Company, p 810). Alcohols, aldehydes and ketones all may be protected by forming respectively, ethers and esters, acetals or ketals by known methods such as

15   those described in McOmie, Plenum Publishing Press, 1973 and Protecting Groups, Ed. Greene, John Wiley & Sons, 1981.

The acids and salts derived from compounds of the invention are readily obtainable from the corresponding esters. Basic saponification with an alkali metal base will provide the acid. For example, an ester of the invention may be dissolved

20   in a polar solvent such as an alkanol, preferably under an inert atmosphere at room temperature, with about a three molar excess of base, for example, lithium hydroxide or potassium hydroxide. The solution is stirred for an extended period of time, between 15 and 20 hours, cooled, acidified and the hydrolysate recovered by conventional means.

25   The amide may be formed by any appropriate amidation means known in the art from the corresponding esters or carboxylic acids. One way to prepare such compounds is to convert an acid to an acid chloride and then treat that compound with ammonium hydroxide or an appropriate amine. For example, the ester is treated with an alcoholic base solution such as ethanolic KOH (in approximately a

10% molar excess) at room temperature for about 30 minutes. The solvent is removed and the residue taken up in an organic solvent such as diethyl ether, treated with a dialkyl formamide and then a 10-fold excess of oxalyl chloride. This is all effected at a moderately reduced temperature between about -10 degrees and

5   +10 degrees C. The last mentioned solution is then stirred at the reduced temperature for 1-4 hours, preferably 2 hours. Solvent removal provides a residue which is taken up in an inert organic solvent such as benzene, cooled to about 0 degrees C and treated with concentrated ammonium hydroxide. The resulting mixture is stirred at a reduced temperature for 1 - 4 hours. The product is

10   recovered by conventional means.

Alcohols are made by converting the corresponding acids to the acid chloride with thionyl chloride or other means (J. March, "Advanced Organic Chemistry", 2nd Edition, McGraw-Hill Book Company), then reducing the acid chloride with sodium borohydride (March, Ibid, pg. 1124), which gives the

15   corresponding alcohols. Alternatively, esters may be reduced with lithium aluminum hydride at reduced temperatures. Alkylating these alcohols with appropriate alkyl halides under Williamson reaction conditions (March, Ibid, pg. 357) gives the corresponding ethers. These alcohols can be converted to esters by reacting them with appropriate acids in the presence of acid catalysts or dicyclohex-

20   ylcarbodiimide and dimethylaminopyridine.

Aldehydes can be prepared from the corresponding primary alcohols using mild oxidizing agents such as pyridinium dichromate in methylene chloride (Corey, E. J., Schmidt, G., Tet. Lett., 399, 1979), or dimethyl sulfoxide/oxalyl chloride in methylene chloride (Omura, K., Swern, D., Tetrahedron, 1978, 34, 1651).

25   Ketones can be prepared from an appropriate aldehyde by treating the aldehyde with an alkyl Grignard reagent or similar reagent followed by oxidation.

Acetals or ketals can be prepared from the corresponding aldehyde or ketone by the method described in March, Ibid, p 810.

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The compounds of the invention which are in accordance with **Formula 4** can be prepared in analogy to the synthetic routes described in **Reaction Schemes 1** and **1a**. In order to obtain these compounds of the invention, a halogenated benzene derivative, such as bromobenzene, iodobenzene (or a subtituted derivative

5   thereof where the substituent is R_2) is reacted with the (trimethylsilyl)ethynylaryl compounds of **Formula 9 (Scheme 1)** or of **Formula 14 (Scheme 1a)**.

Referring now to **Reaction Scheme 2**, a synthetic route is described to obtain compounds of the invention in accordance with **Formula 1**.

R'_3

$(R_3)_o$ — Br

Formula 10

→ 2 equivalents of tBuLi

THF, - 78° C

R'_3

$(R_3)_o$ — Li

Formula 16

$(R_4)_2SiCl_2$
Formula 17

$$\left\{ \quad R'_3 \atop (R_3)_o \text{—} Si(R_4)_2Cl \quad (R_2)_m \right\}$$

Formula 18

Br-Y(R$_2$)-CH$_2$OSiPh$_2$tBu
Formula 8

→ 2 equivalents of tBuLi

THF, - 78° C

$$\left\{ \text{Li-Y(R}_2)\text{-CH}_2\text{OSiPh}_2\text{tBu} \atop \textbf{Formula 19} \right\}$$

Formula 18 + Formula 19

THF, - 78° C

R'_3

$(R_3)_o$ —Si(R$_4$)$_2$-Y(R$_2$)-CH$_2$OSiPh$_2$tBu

$(R_2)_m$

Formula 20

REACTION SCHEME 2

43

R'3

(R3)o∿∿

X

(R2)m

Si(R4)2-Y(R2)-CH2OSiPh2tBu
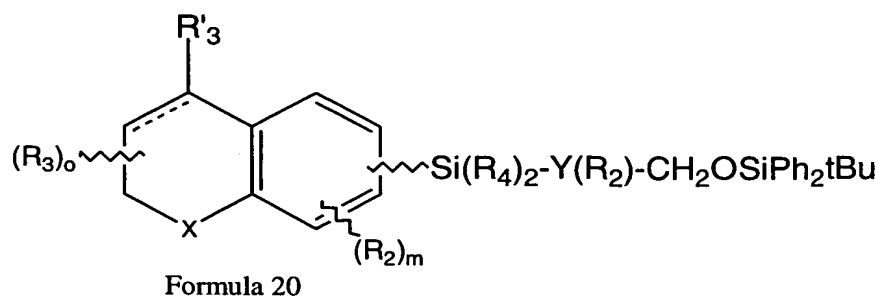
Formula 20

(1) tetrabutylammonium fluoride

(2) MnO2

(3) MnO2, NaCN, EtOH

R'3

(R3)o∿∿

X

(R2)m

Si(R4)2-Y(R2)-COOEt
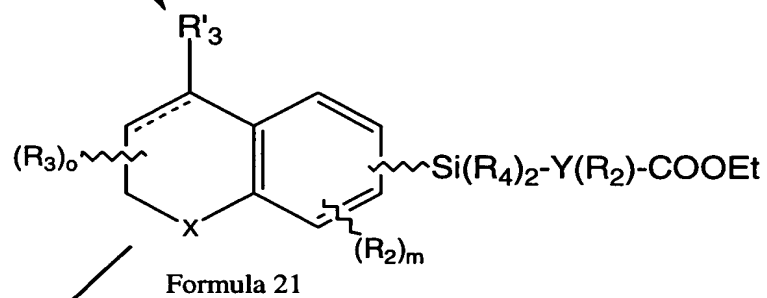
Formula 21

HOMOLOGS AND DERIVATIVES

REACTION SCHEME 2 (Continued)

The starting compounds utilized in **Reaction Scheme 2** are the condensed cyclic bromoaryl compounds of **Formula 10**, which have been described above in connection with **Reaction Scheme 1**, and **1a**. The bromo aryl compounds of **Formula 10** are converted into an organometallic, preferably, organolithium

5   reagent, as is shown in **Scheme 2**. Exchange of the bromine (or of iodine if an iodoaryl reagent is used) with lithium is conducted under conditions normally practiced in the art, typically with two equivalents of *tert*-butyl lithium, in an ethereal reagent (THF) in the cold, typically -78 °F. The resulting condensed cyclic aryl lithium reagent of **Formula 16** is then reacted with a

10 dialkyldichlorosilane, alkylphenyldichlorosilane or diphenyldichlorosilane reagent of **Formula 17**. The R_4 groups in **Formula 17** have the same definition as in connection with **Formulas 1 - 4**. The dialkyldichlorosilane, alkylphenyldichlorosilane or diphenyldichlorosilane reagents are available commercially, or can be prepared in accordance with known procedures within the

15 skill of the ordinary practitioner in the field.

As is shown in **Reaction Scheme 2**, with the bromoaryl compound of **Formula 10** the $(R_4)_2SiCl_2$ reagent forms an aryl dialkylchlorosilane of **Formula 18**. The latter is typically not isolated, but used without isolation to react with an organolithium compound of **Formula 19** that is also prepared by bromine - lithium

20 exchange from the (bromoaryl)methyl *t*-butyldiphenylsilyl ether of **Formula 8**, described above in connection with **Reaction Scheme 1**. The (aryl)methyl *t*-butyldiphenylsilyl ether lithium reagent of **Formula 19** is also typically not isolated before reacting it with the reagent of **Formula 18**. This is indicated in the reaction scheme by placing the reagents of **Formulas 18** and **19** in large square

25 brackets.

The product of the reaction between the aryl dialkylchlorosilane of **Formula 18** and the (aryl)methyl *t*-butyldiphenylsilyl ether lithium reagent of **Formula 19** is the diarylsilane compound of **Formula 20** that still has the *tert*-butyldiphenylsilyl protecting group on the primary alcohol function. This is

removed by treatment with tetrabutylammonium fluoride, and the resulting primary alcohol can be oxidized to the ester stage (**Formula 21**) in analogy to the reactions described in connection with **Reaction Scheme 1**. The diarylsilane compounds of **Formula 21** are within the scope of the invention, particularly within the scope of
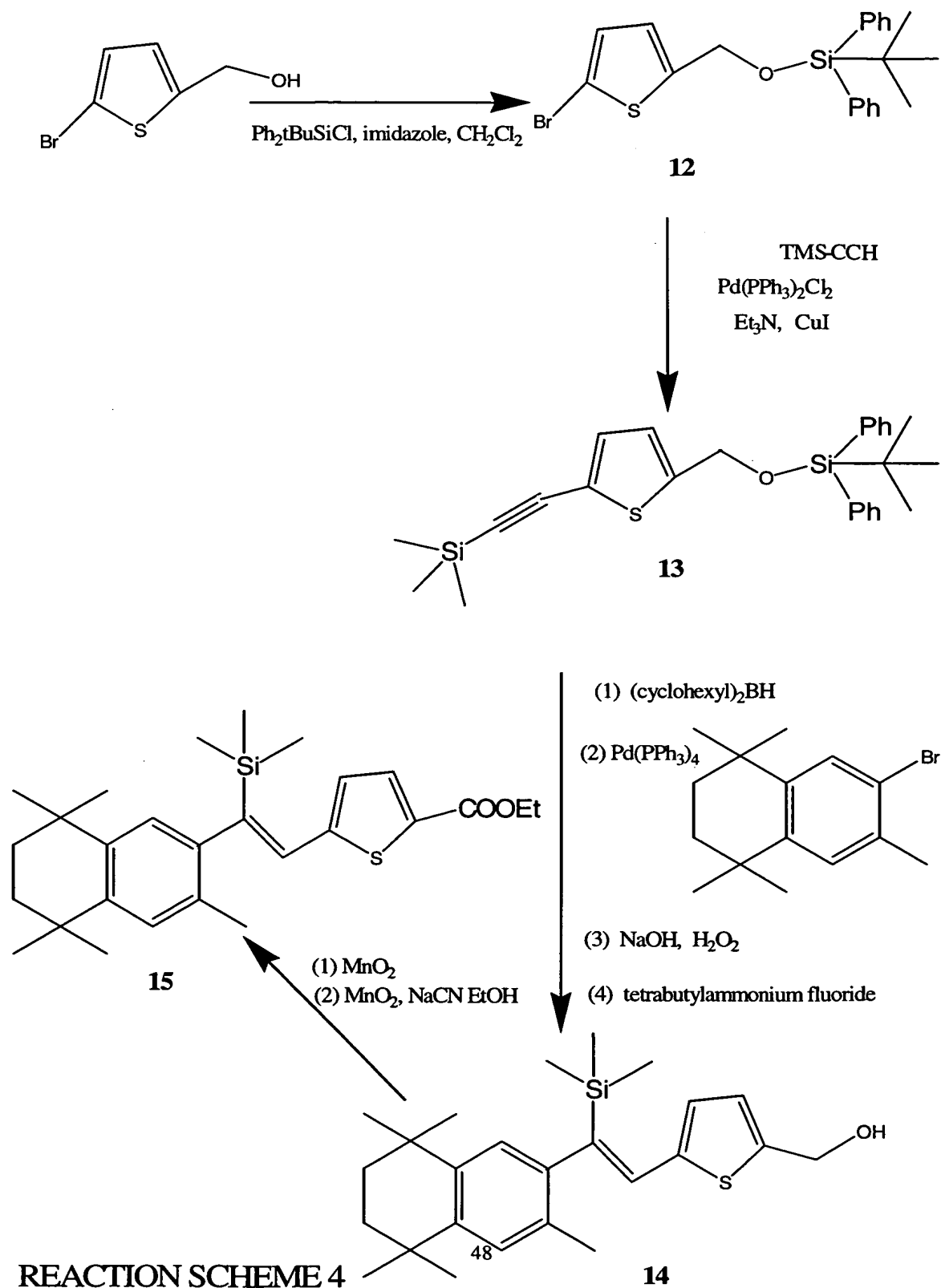
5   **Formula 1** and can be converted into further homologs and derivatives, as described above.   A particularly preferred step of such conversion is saponifaction of the ester group with base to provide the free carboxylic acids (or salts thereof) of the invention.

The diarylsilane compounds of **Formula 2** can be prepared in analogy to

10   the preparation of the diarylsilane compounds of **Formula 1**. A starting material in this synthetic route is a halogenated benzene derivative, such as bromobenzene, iodobenzene or a subtituted derivative thereof where the substituent is R_2.

REACTION SCHEME 3

12

TMS-CCH

Pd(PPh₃)₂Cl₂

Et₃N, CuI

13

(1) (cyclohexyl)₂BH

(2) Pd(PPh₃)₄

(3) NaOH, H₂O₂

(4) tetrabutylammonium fluoride

15

(1) MnO₂
(2) MnO₂, NaCN EtOH

14
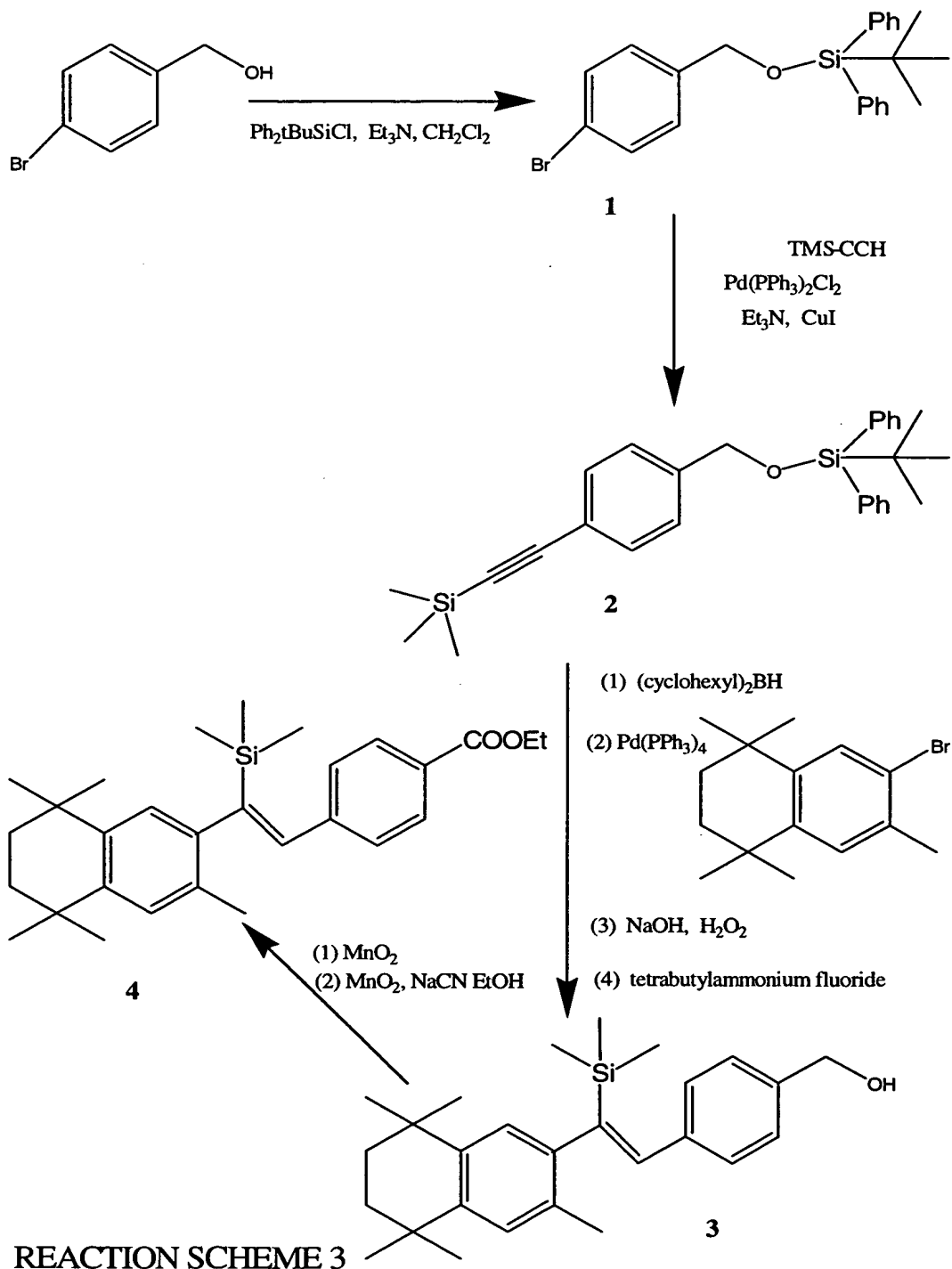
REACTION SCHEME 4

Reaction Schemes 3 and 4 illustrate the synthesis of certain exemplary compounds of the invention. The synthetic processes illustrated in these two schemes are described in detail in the section titled "Specific Chemical Examples" below.

SPECIFIC CHEMICAL EXAMPLES

4-Bromobenzyl tert-butyldiphenylsilyl ether (Compound 1)

Tert-butyldiphenylsilyl chloride (10.4 mL, 40.1 mmol) was added to a solution of 4-bromobenzyl alcohol (5.0 g, 26.7 mmol) and 50 mL of dichloromethane. The solution was treated with triethylamine (3.72 mL, 26.7 mmol) and (dimethylamino)pyridine (163 mg, 1.34 mmol) and stirred overnight at room temperature. The solution was diluted with 300 mL of dichloromethane and washed with 50 mL of 10% aqueous HCl. The layers were separated and the aqueous layer was extracted with 50 mL of dichloromethane. The combined organic extracts were washed with brine, and dried (MgSO$_4$), and filtered, and the solvents were removed *in vacuo*. The residue was filtered through a plug (6@ X 2@) of silica gel using a solution of 97 % hexane/ethyl acetate. After removal of the solvent the residue was heated under vacuum (3 torr) to 170 °C for 1 hour to remove a low-boiling impurity. The remaining material is the title compound. PNMR (300 MHz, CDCl$_3$) • 1.09 (s, 9 H), 4.70 (s, 2 H), 7.20 (d, 2 H, *J* = 7.9 Hz), 7.35-7.45 (m, 8 H), 7.65 (overlapping ds, 4 H).

4-[(trimethylsilyl)ethynyl]benzyl tert-butyldiphenylsilyl ether (Compound 2)

A 25 mL round bottom flask was flame-dried under high vacuum. The vacuum was broken by the addition of dry argon, and the flask was allowed to cool to room temperature. The flask was charged with 2.0 g (4.70 mmol) of 4-bromobenzyl tert-butyldiphenylsilyl ether (Compound 1), 2.0 mL (14.1 mmol) of

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(trimethylsilyl)acetylene, and 16.5 mL of triethylamine. The solution was purged with argon for 15 minutes and bis(triphenylphosphine)palladium (II) chloride (83 mg, 0.12 mmol) and copper (I) iodide (22 mg, 0.12 mmol) were added and the solution stirred at ambient temperature for 3 days. The solution was poured into a separatory funnel containing water and ether. The layers were separated and the aqueous layer was extracted 3 times with ether. The combined ether layers were washed once with brine, and dried over magnesium sulfate, and the solvents were removed under reduced pressure. The residue was purified by distillation (bp = 180° B 185 °C, 1 torr) to give the title compound.

PNMR (300 MHz, CDCl₃) • 0.23 (s, 9 H), 1.09 (s, 9 H), 4.73 (s, 2 H), 7.23 (d, 2 H, J = 7.9 Hz), 7.31-7.45 (m, 8 H), 7.65 (overlapping ds, 4 H).

(Z)-4-[2-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]benzyl alcohol (**Compound 3**) **General Procedure A** A 3-neck 25 mL round bottom flask was fitted with a reflux condenser, and flame-dried under high vacuum. The vacuum was broken by the addition of dry argon (3x), and the flask was allowed to cool to room temperature. The flask was charged with 0.5 mL (1.0 mmol) of borane-methyl sulfide and THF (0.3 mL) and cooled to 0 °C. The solution was treated with 0.20 mL (2 mmol) of cyclohexene and stirred at 0 °C for 1 hour. Neat 4-[(trimethylsilyl)ethynyl]benzyl *tert*-butyldiphenylsilyl ether (**Compound 2**, 443 mg, 1 mmol) was added and, after 15 minutes the solution was warmed to room temperature and stirred for 2.25 hours. In a second flask was prepared a solution of tetrakis(triphenylphosphine)palladium (0) (58 mg, 0.05 mmol) and 2-bromo-3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalene (1.26 g, 4.5 mmol) in 5 mL of THF, which was purged with argon for 10 minutes. The solvents in the first flask were removed under high vacuum, and the residue dissolved in 1 mL of THF and 1 mL of 2 M aqueous NaOH, and the resulting solution was purged with argon for 10 minutes. A 1 mL aliquot of the solution

from the second flask was added to the first flask, and the reaction was protected from light and refluxed for 5 hours. The reaction was cooled to room temperature and treated with 2 M NaOH (1 mL) and 30 % hydrogen peroxide (0.4 mL). The solution was poured into a separatory funnel containing water and pentane. The layers were separated and the aqueous layer was extracted 3 times with pentane. The combined organic layers were washed once with brine, and dried over magnesium sulfate, and the solvents were removed under reduced pressure. The residue was partially purified by silica gel chromatograhy (99:1, hexane:ethyl acetate). The later fractions were combined and concentrated under reduced pressure. The residue (203 mg) was dissolved in 3.2 mL of THF and treated with 313 mg of tetrabutylammonium flouride (Tbaf) adsorbed onto silica gel (1.6 mmol flouride per gram). The suspension was stirred for 5 hours at room temperature and then the silica gel was washed with ether, and the separated ether extracts were dried over magnesium sulfate. The filtered solvents were removed under reduced pressure and the residue purified by silica gel chromatography (4:1, hexane:ethyl acetate) to give the title compound.

PNMR (300 MHz, CDCl$_3$) - 0.10 (s, 9 H), 1.29 (s, 12 H), 1.68 (s, 4 H), 2.24 (s, 3 H), 4.72 (s, 2 H), 6.87 (s, 1 H), 7.07 (s, 1 H), 7.17 (s, 1 H), 7.35 (s, 4 H).

Ethyl (Z)-4-[2-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]benzoate (Compound 4) General Procedure B

Manganese dioxide (265 mg, 2.96 mmol) was added to a solution of (Z)-4-[2-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]benzyl alcohol (Compound 3, 60 mg, 0.15 mmol) and 3.65 mL of hexane. The solution was stirred at room temperature for 16 hours, the manganese dioxide filtered off, and the hexane removed in vacuo. The residue was dissolved in 2 mL of ethanol and treated with sodium cyanide (37.5 mg, 0.77 mmol) and acetic acid (13.7 mg, 0.23 mmol). After 15 minutes, the solution was

treated with 265 mg (3.0 mmol) of manganese dioxide. The suspension was stirred at room temperature for 6 hours and the manganese dioxide removed by filtration. The solution was poured into a separatory funnel containing water and ether. The layers were separated and the aqueous layer was extracted 3 times with ether. The combined organic layers were washed once with brine, and dried over magnesium sulfate, and the solvents were removed under reduced pressure. The residue was purified by silica gel chromatograhy (97:3, hexane:ethyl acetate) to give the title compound. PNMR (300 MHz, $CDCl_3$) - 0.11 (s, 9 H), 1.28 (s, 12 H), 1.41 (t, 3 H, J = 7.1 Hz), 1.68 (s, 4 H), 2.23 (s, 3 H), 4.39 (q, 2 H, J = 7.1 Hz), 6.86 (s, 1 H), 7.08 (s, 1 H), 7.17 (s, 1 H), 7.41 (d, 2 H, J = 8.5 Hz), 8.03 (d, 2 H, J = 8.5 Hz).

(Z)-4-[2-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]benzoic Acid   (Compound 5)   General Procedure C

To a solution of ethyl (Z)-4-[2-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]benzoate (Compound 4, 0.034 g, 0.076 mmol) and 2 mL of ethyl alcohol was added aqueous 1 N KOH (0.5 mL). The resulting solution was heated in an 50 °C bath until the hydrolysis reaction was completed, as judged by thin layer chromatography. The solution was cooled to room temperature, diluted with water and washed once with 1:1 ether:hexane solution, and the layers were separated. The aqueous layer was acidified with 1 N aqueous HCl and the product extracted 3 times with ethyl acetate. The combined organic extracts were washed with brine, and dried over $MgSO_4$, and filtered, and the solvents were removed *in vacuo* to give the title compound as a white solid. PNMR (300 MHz, $CDCl_3$) • - 0.09 (s, 9 H), 1.28 (s, 12 H), 1.68 (s, 4 H), 2.24 (s, 3 H), 6.86 (s, 1 H), 7.08 (s, 1 H), 7.18 (s, 1 H), 7.46 (d, 2 H, J = 8.1 Hz), 8.11 (d, 2 H, J = 8.1 Hz).

(Z)-4-[2-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-
(trimethylsilyl)vinyl]benzyl alcohol **(Compound 6)**

Following General Procedure A, 4-[(trimethylsilyl)ethynyl]benzyl *tert*-
butyldiphenylsilyl ether **(Compound 2**, 0.89g, 2.0 mmol) and 2-bromo-5,5,8,8-
tetramethyl-5,6,7,8-tetrahydronaphthalene (0.60 g, 2.25 mmol) were coupled to
give the title compound. 2-bromo-5,5,8,8-tetramethyl-5,6,7,8-
tetrahydronaphthalene can be prepared in accordance with the procedure set forth
in *J. Med. Chem.* 34:2930-41 (1994). The pentamethyl derivative thereof can be
prepared in accordance with the same procedure.

PNMR (300 MHz, CDCl$_3$) - 0.05 (s, 9 H), 1.30 (s, 6 H), 1.32 (s, 6 H), 1.70 (s, 4
H), 4.72 (s, 2 H), 6.97 (dd, 1 H, J = 2.0, 8.1 Hz), 7.10 (d, 1 H, J = 2.0 Hz), 7.24 (d,
1 H, J = 8.1 Hz), 7.28 (s, 1 H), 7.33 (s, 4 H).

Ethyl (Z)-4-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-
(trimethylsilyl)vinyl]benzoate **(Compound 7)**

Following General Procedure B, (Z)-4-[2-(5,5,8,8-tetramethyl-5,6,7,8-
tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]benzyl alcohol **(Compound 6**,
0.50 g, 1.3 mmol) was oxidized to give the title compound.

PNMR (300 MHz, CDCl$_3$) • - 0.05 (s, 9 H), 1.29 (s, 6 H), 1.31 (s, 6 H), 1.41 (t, 3
H, J = 7.1 Hz), 1.69 (s, 4 H), 4.39 (d, 2 H, J = 7.1 Hz), 6.95 (dd, 1 H, J = 2.0, 8.1
Hz), 7.09 (d, 1 H, J = 2.0 Hz), 7.24 (d, 1 H, J = 8.1 Hz), 7.27 (s, 1 H), 7.38 (d, 2 H,
J = 8.3 Hz), 8.02 (d, 2 H, J = 8.3 Hz).

(Z)-4-[2-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-
(trimethylsilyl)vinyl]benzoic acid **(Compound 8)**

Following General Procedure C, ethyl (Z)-4-[2-(5,5,8,8-tetramethyl-5,6,7,8-
tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]benzoate **(Compound 7**, 0.205
g, 0.47 mmol) was hydrolyzed to give the title compound.

PNMR (300 MHz, CDCl$_3$) - 0.04 (s, 9 H), 1.29 (s, 6 H), 1.32 (s, 6 H), 1.70 (s, 4 H), 6.97 (dd, 1 H, J = 2.0, 8.1 Hz), 7.09 (d, 1 H, J = 2.0 Hz), 7.25 (d, 1 H, J = 8.1 Hz), 7.29 (s, 1 H), 7.43 (d, 2 H, J = 8.1 Hz), 8.09 (d, 2 H, J = 8.1 Hz).

<u>Ethyl 4-[(trimethylsilyl)ethynyl]benzoate</u>   **(Compound 9)**

A resealable tube was flame-dried under high vacuum. The vacuum was broken by the addition of dry argon, and the flask was allowed to cool to room temperature. The flask was charged with 5.0 g (18.1 mmol) of ethyl 4-bromobenzoate, 7.7 mL (54.3 mmol) of (trimethylsilyl)acetylene, and 65 mL of diethylamine. The solution was purged with argon for 15 minutes and bis(triphenylphosphine)palladium (II) chloride (320 mg, 0.45 mmol) and copper (I) iodide (87 mg, 0.45 mmol) were added, the tube sealed, and the solution stirred at 55 °C for 3 days. The solution was poured into a separatory funnel containing water and ether. The layers were separated and the aqueous layer was extracted 3 times with ether. The combined ether layers were washed once with brine, and dried over magnesium sulfate, and the solvents were removed under reduced pressure. The residue was purified by silica gel chromatography (95:5. hexane:ethyl acetate) to give the title compound.

PNMR (300 MHz, CDCl$_3$) • 0.26 (s, 9 H), 1.39 (t, 3 H, J = 7.1 Hz), 4.36 (q, 2 H, J = 7.1 Hz), 7.51 (d, 2 H, J = 8.6 Hz), 7.97 (d, 2 H, J = 8.6 Hz).

<u>Ethyl (Z)-4-[2-(2,2,4,4-tetramethylchroman-6-yl)-2-(trimethylsilyl)vinyl]benzoate</u>
(Compound 10)

Following General Procedure A, ethyl 4-[(trimethylsilyl)ethynyl]benzoate **(Compound 9**, 0.51 g, 2.0 mmol) and 6-bromo-2,2,4,4-tetramethylchroman (0.57 g, 2.25 mmol) were coupled to give the title compound.

PNMR (300 MHz, CDCl$_3$) • - 0.06 (s, 9 H), 1.36 (s, 6 H), 1.37 (s, 6 H), 1.39 (t, 3 H, J = 7.1 Hz), 1.85 (s, 2 H), 4.38 (q, 2 H, J = 7.1 Hz), 6.75 (d, 1 H, J = 8.3 Hz),

6.94 (dd, 1 H, J = 2.3, 8.3 Hz), 7.07 (s, 1 H), 7.26 (d, 1 H, J = 2.3 Hz), 7.38 (d, 2 H, J = 7.9 Hz), 8.02 (d, 2 H, J = 7.9 Hz).

(Z)-4-[2-(2,2,4,4-Tetramethylchroman-6-yl)-2-(trimethylsilyl)vinyl]benzoic acid
(Compound 11)

Following General Procedure C, ethyl (Z)-4-[2-(2,2,4,4-tetramethylchroman-6-yl)-2-(trimethylsilyl)vinyl]benzoate (**Compound 10**, 0.48 g, 1.1 mmol) was hydrolyzed to give the title compound.

PNMR (300 MHz, CDCl₃) • - 0.04 (s, 9 H), 1.37 (s, 6 H), 1.39 (s, 6 H), 1.86 (s, 2 H), 6.76 (d, 1 H, J = 8.3 Hz), 6.94 (dd, 1 H, J = 2.2, 8.3 Hz), 7.09 (d, 1 H, J = 2.2 Hz), 7.29 (s, 1 H), 7.44 (d, 2 H, J = 8.2 Hz), 8.11 (d, 2 H, J = 8.2 Hz).

(5-Bromothiophen-2-yl)methyl *tert*-butyldiphenylsilyl ether **(Compound 12)**

Tert-butyldiphenylsilyl chloride (7.8 mL, 30.1 mmol) was added to a solution of 5-bromo(thiophen-2-yl)methyl alcohol (4.9 g, 25.1 mmol) and 9.7 mL of dimethylformamide. The solution was treated with imidazole (4.29 g, 62.8 mmol) and stirred overnight at room temperature. The solution was diluted with ether and washed with 2 % aqueous HCl. The layers were separated and the aqueous layer was extracted with ether. The combined organic extracts were washed with brine, and dried (MgSO₄), and filtered, and the solvents were removed *in vacuo*. The residue was purified by silica gel chromatography (hexane) to produce the title compound.

PNMR (300 MHz, CDCl₃) • 1.10 (s, 9 H), 4.80 (s, 2 H), 6.56 (d, 1 H, J = 2.4 Hz), 6.88 (d, 1 H, J = 2.4 Hz), 7.38-7.50 (m, 8 H), 7.70 (m, 4 H).

5-[(Trimethylsilyl)ethynyl]thiophen-2-ylmethyl *tert*-butyldiphenylsilyl ether
(Compound 13)

A round bottom flask was flame-dried under high vacuum. The vacuum was broken by the addition of dry argon, and the flask was allowed to cool to room temperature. The flask was charged with 2.16 g (5.0 mmol) of (5-bromothiophen-2-yl)methyl *tert*-butyldiphenylsilyl ether (**Compound 12**), 2.12 mL (15 mmol) of (trimethylsilyl)acetylene, and 17.5 mL of triethylamine. The solution was purged with argon for 15 min and bis(triphenylphosphine)palladium (II) chloride (88 mg, 0.125 mmol) and copper (I) iodide (24 mg, 0.125 mmol) were added and the solution stirred at ambient temperature for 3 days. The solution was poured into a separatory funnel containing water and ether. The layers were separated and the aqueous layer was extracted 3 times with ether. The combined ether layers were washed once with brine, and dried over magnesium sulfate, and the solvents were removed under reduced pressure. The residue was purified by silica gel chromatography (hexane) to give the title compound.

PNMR (300 MHz, CDCl$_3$) • 0.25 (s, 9 H), 1.08 (s, 9 H), 4.83 (s, 2 H), 6.63 (d, 1 H, J = 3.8 Hz), 7.06 (d, 1 H, J = 3.8 Hz), 7.41 (m, 8 H), 7.68 (overlapping ds, 4 H).

(Z)-5-[2-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]thiophene-2-ylmethyl alcohol (**Compound 14**)

Following General Procedure A, 5-[(trimethylsilyl)ethynyl]thiophen-2-ylmethyl *tert*-butyldiphenylsilyl ether (**Compound 13**, 0.75 g, 1.8 mmol) and 2-bromo-3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalene (0.45 g, 1.67 mmol) were coupled to give the title compound.

PNMR (300 MHz, CDCl$_3$) • 0.2 (s, 9 H), 1.42 (s, 6 H), 1.43 (s, 6 H), 1.83 (s, 4 H), 2.34 (s, 3 H), 4.95 (s, 2 H), 6.97 (s, 1 H), 7.04 (s, 2 H), 7.19 (s, 1 H), 7.21 (s, 1 H).

Ethyl (Z)-5-[2-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]thiophene-2-carboxylate (**Compound 15**)

Following General Procedure B, (Z)-5-[2-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]thiophene-2-ylmethyl alcohol (**Compound 14**, 0.088 g, 0.213 mmol) was oxidized to give the title compound. PNMR (300 MHz, CDCl₃) • - 0.029 (s, 9 H), 1.26 (s, 6 H), 1.27 (s, 6 H), 1.39 (t, 3 H, J = 7.1 Hz), 1.67 (s, 4 H), 2.17 (s, 3 H), 4.35 (q, 2 H, J = 7.1 Hz), 6.80 (s, 1 H), 7.00 (d, 1 H, J = 3.8 Hz), 7.02 (s, 1 H), 7.06 (s, 1 H), 7.69 (d, 1 H, J = 3.8 Hz).

(Z)-5-[2-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]thiophene-2-carboxylic acid   (**Compound 16**)

Following General Procedure C, ethyl (Z)-5-[2-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]thiophene-2-carboxylate (**Compound 15**, 0.050 g, 0.11 mmol) was hydrolyzed to give the title compound. PNMR (300 MHz, CDCl₃) • 0.04 (s, 9 H), 1.26 (s, 6 H), 1.27 (s, 6 H), 1.67 (s, 4 H), 2.18 (s, 3 H), 7.02 (s, 1 H), 7.04 (s, 1 H), 7.05 (d, 1 H, J = 4.1 Hz), 7.26 (s, 1 H), 7.79 (d, 1 H, J = 4.1 Hz).

(Z)-5-[2-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]thiophene-2-ylmethyl alcohol (**Compound 17**)

Following General Procedure A, 5-[(trimethylsilyl)ethynyl]thiophen-2-ylmethyl *tert*-butyldiphenylsilyl ether (**Compound 13**, 0.75 g, 1.8 mmol) and 2-bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalene (0.46 g, 1.62 mmol) were coupled to give the title compound.
PNMR (300 MHz, CDCl₃) • 0.2 (s, 9 H), 1.42 (s, 6 H), 1.43 (s, 6 H), 1.83 (s, 4 H), 4.95 (s, 2 H), 6.97 (s, 1 H), 7.04 (s, 2 H), 7.19 (s, 1 H), 7.21 (s, 1 H).

Ethyl (Z)-5-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]thiophene-2-carboxylate (**Compound 18**)

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Following General Procedure B, (Z)-5-[2-(3,5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]thiophene-2-ylmethyl alcohol (**Compound 17**, 0.30 g, 0.753 mmol) was oxidized to give the title compound. PNMR (300 MHz, CDCl$_3$) • - 0.029 (s, 9 H), 1.26 (s, 6 H), 1.27 (s, 6 H), 1.39 (t, 3 H, J = 7.1 Hz), 1.67 (s, 4 H), 4.35 (q, 2 H, J = 7.1 Hz), 6.80 (s, 1 H), 7.00 (d, 1 H, J = 3.8 Hz), 7.02 (s, 1 H), 7.06 (s, 1 H), 7.69 (d, 1 H, J = 3.8 Hz).

(Z)-5-[2-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]thiophene-2-carboxylic Acid (**Compound 19**)

Following General Procedure C, ethyl (Z)-5-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trimethylsilyl)vinyl]thiophene-2-carboxylate (**Compound 18**, 0.125 g, 0.284 mmol) was hydrolyzed to give the title compound. PNMR (300 MHz, CDCl$_3$) • 0.04 (s, 9 H), 1.26 (s, 6 H), 1.27 (s, 6 H), 1.67 (s, 4 H), 7.02 (s, 1 H), 7.04 (s, 1 H), 7.05 (d, 1 H, J = 4.1 Hz), 7.26 (s, 1 H), 7.79 (d, 1 H, J = 4.1 Hz).

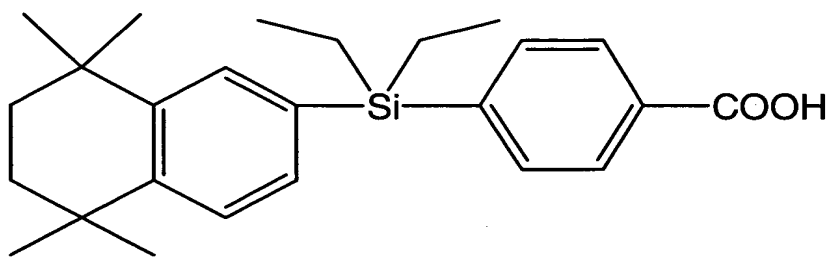4-[Diethyl(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl]silylbenzyl alcohol (**Compound 20**)

To a -78 °C solution of 2-bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalene (1.34 g, 5.0 mmol) in 6.9 mL of THF was added *n*-butyllithium (1.6 M, 3.13 mL, 5.0 mmol). After ten minutes, the solution was added via canula to a B78 °C solution of diethyldichlorosilane (0.61 mL, 5.0 mmol) and THF (4.4 mL) and stirring continued for 1 hour. In a second flask containing 4-bromobenzyl *tert*-butyldiphenylsilyl ether (**Compound 1**, 3.19 g, 7.5 mmol) and THF (2 mL) at -78 °C was added *n*-butyllithium (1.6 M, 4.69 mL, 7.5 mmol). After ten minutes, the contents of the second flask were added via canula to the first flask. After 30 minutes at -78 °C, the reaction was quenched by the addition of 5 mL of saturated aqueous NH$_4$Cl. The solution was poured into a separatory funnel

containing water and hexane. The layers were separated and the aqueous layer was extracted 3 times with hexane. The combined organic layers were washed once with brine, and dried over magnesium sulfate, and the solvents were removed under reduced pressure. The residue was dissolved in 20 mL of THF and treated with 3.2 g of tetrabutylammonium flouride (Tbaf) adsorbed onto silica gel (1 - 1.6 mmol flouride per gram). The suspension was stirred for 5 hours at room temperature and then the silica gel was washed with ether, and the separated ether extracts were dried over magnesium sulfate. The filtered solvents were removed under reduced pressure and the residue purified by silica gel chromatography (9:1, hexane:ethyl acetate) to give the title compound.

PNMR (300 MHz, CDCl$_3$) • 0.91-1.06 (m, 10 H), 1.25 (s, 6 H), 1.28 (s, 6 H), 1.68 (s, 4 H), 4.70 (s, 2 H), 7.22-7.25 (overlapping ds, 2 H), 7.35 (d, 2 H, J = 8.1 Hz), 7.44 (s, 1 H), 7.53 (d, 1 H, J = 8.1 Hz).

Ethyl 4-[diethyl(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl]silylbenzoate (Compound 21)

Following General Procedure B, 4-[diethyl(5,5,8,8-tetramethyl-5,6,7,8-
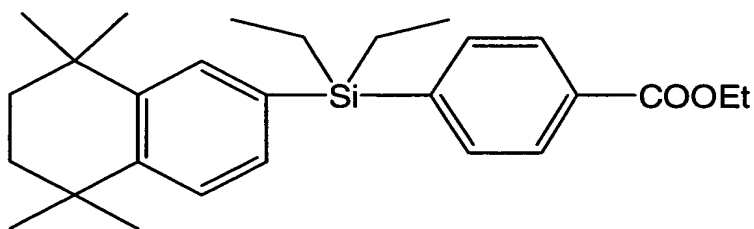
Compound 21

tetrahydronaphthalen-2-yl]silylbenzyl alcohol (Compound 20, 1.25 g, 3.30 mmol) was oxidized to give the title compound.

PNMR (300 MHz, CDCl₃) • 0.99-1.09 (m, 10 H), 1.24 (s, 6 H), 1.28 (s, 6 H), 1.39 (t, 3 H, J = 7.1 Hz), 1.67 (s, 4 H), 4.37 (q, 2 H, J = 7.1 Hz), 7.22-7.29 (overlapping ds, 2 H), 7.42 (s, 1 H), 7.60 (d, 2 H, J = 8.1 Hz), 8.00 (d, 1 H, J = 8.1 Hz).

4-[Diethyl(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl]silylbenzoic Acid (Compound 22)

Compound 22

Following General Procedure C, ethyl 4-[diethyl(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl]silylbenzoate (Compound 21, 0.650 g, 1.54 mmol) was hydrolyzed to give the title compound.

PNMR (300 MHz, CDCl₃) • 0.99-1.10 (m, 10 H), 1.25 (s, 6 H), 1.28 (s, 6 H), 1.68 (s, 4 H), 7.22-7.30 (overlapping ds, 2 H), 7.42 (s, 1 H), 7.64 (d, 2 H, J = 8.1 Hz), 8.07 (d, 1 H, J = 8.1 Hz).
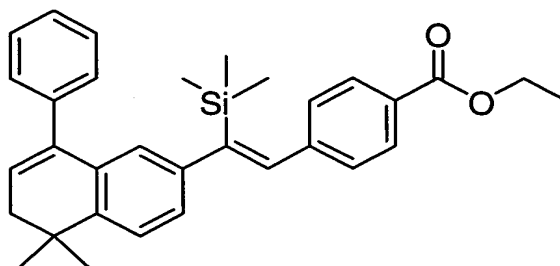
4-[(Z)-(5,5-Dimethyl-8-p-tolyl-5,6-dihydronaphthalen-2-yl)trimethylsilanylvinyl]-benzyl Alcohol (Compound 23).
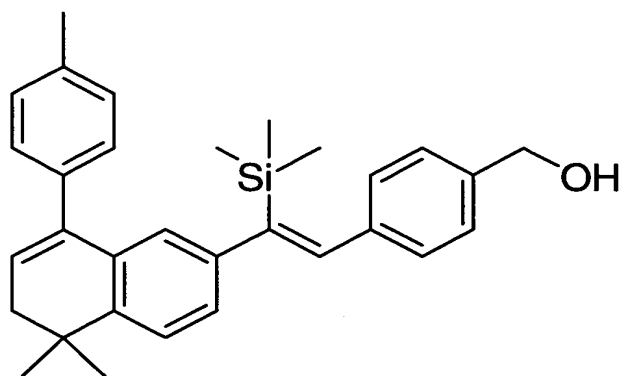
Compound 23

Following General Procedure A, 4-[(trimethylsilyl)ethynyl]benzyl *tert*-butyldiphenylsilyl ether and 6-bromo-1,1-dimethyl-4-p-tolyl-1,2-dihydronaphthalene (prepared as described in Klein, et al.,U.S. Patent 5,952,345) were coupled to give the title compound **(Compound 23)**. PNMR (300 MHz, CDCl3): δ 0.13 (s, 9 H), 1.47 (s, 6 H), 2.48 (d, J = 4.4 Hz, 2 H), 2.54 (s, 3 H), 4.82 (d, J = 6.1 Hz, 2 H), 6.10 (t, J = 4.4 Hz, 1 H), 7.00 (d, J = 2.2 Hz, 1 H), 7.18 (dd, J = 2.2, 7.9 Hz, 1 H), 7.30-7.45 (m, 10 H).

Ethyl (Z)-4-[(5,5-Dimethyl-8-*p*-tolyl-5,6-dihydronaphthalen-2-yl)trimethylsilanyl-vinyl]benzoate. **(Compound 24)**
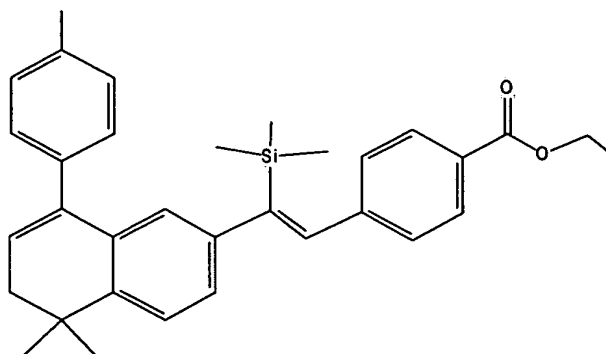
Compound 24

Following General Procedure B, 4-[(Z)-(5,5-dimethyl-8-p-tolyl-5,6-dihydronaphthalen-2-yl)trimethylsilanylvinyl]benzyl alcohol was oxidized to give the title compound **(Compound 24)**. PNMR (300 MHz, CDCl₃): **(Compound 34)** 0.0 (s, 9 H), 1.47 (s, 6 H), 1.53 (t, J = 7.0 Hz, 3 H), 2.48 (d, J = 4.7 Hz, 2 H), 2.54 (s, 3 H), 4.50 (q, J = 7.0 Hz, 2 H), 6.10 (t, J = 4.7 Hz, 1 H), 6.99 (d, J = 2.0 Hz, 1 H), 7.17 (dd, J = 2.0, 7.9 Hz, 1 H), 7.30-7.54 (m, 8 H), 8.11 (d, J = 8.2 Hz, 2 H).
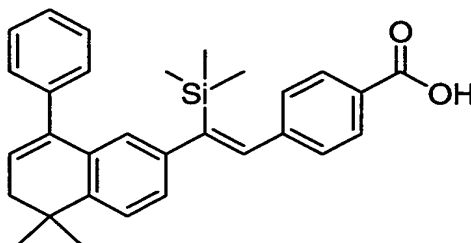
(Z)-4-[(5,5-Dimethyl-8-p-tolyl-5,6-dihydronaphthalen-2-yl)trimethylsilanylvinyl]-
benzoic Acid.(Compound 25)
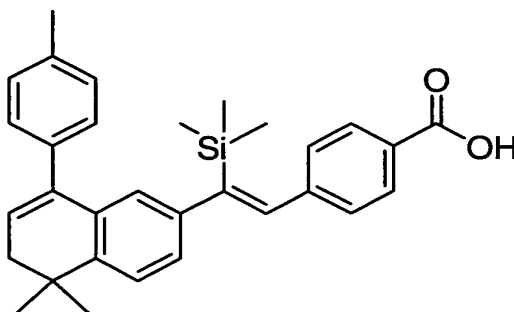
Compound 25

Following General Procedure C, ethyl (Z)-4-[(5,5-dimethyl-8-*p*-tolyl-5,6-dihydronaphthalen-2-yl)trimethylsilanylvinyl]benzoate was hydrolyzed to give the title compound **(Compound 25)**. PNMR (300 MHz, CDCl3): δ 0.0 (s, 9 H), 1.47 (s, 6 H), 2.48 (d, *J* = 4.9 Hz, 2 H), 2.54 (s, 3 H), 6.10 (t, *J* = 4.9 Hz, 1 H), 6.98 (d, *J* = 2.0 Hz, 1 H), 7.16 (dd, *J* = 2.0, 7.9 Hz, 1 H), 7.30-7.60 (m, 8 H), 8.15 (d, *J* = 8.3 Hz, 2 H).

(Z)-{4-(5,5-Dimethyl-8-phenyl-5,6-dihydronaphthalen-2-yl)trimethylsilanylvinyl]-benzyl Alcohol.(**Compound 26**).
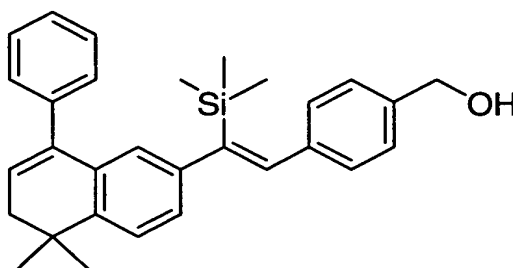
Compound 26

Following General Procedure A, 4-[(trimethylsilyl)ethynyl]benzyl *tert*-butyldiphenylsilyl ether and 6-bromo-1,1-dimethyl-4-phenyl-1,2-dihydronaphthalene (which can be prepared by the procedure described in Klein, et al.,U.S. Patent 5,952,345) were coupled to give the title compound (**Compound 26**). PNMR (300 MHz, CDCl3): δ 0.0 (s, 9 H), 1.49 (s, 6 H), 2.50 (d, *J* = 4.4 Hz, 2 H), 4.80 (d, *J* = 5.7 Hz, 2 H), 6.13 (t, *J* = 4.4 Hz, 1 H), 6.98 (d, *J* = 2.2 Hz, 1 H), 7.21 (dd, *J* = 2.2, 7.9 Hz, 1 H), 7.31-7.60 (m, 7 H).

<u>Ethyl (Z)-4-[(5,5-Dimethyl-8-phenyl-5,6-dihydronaphthalen-2-yl)trimethylsilanyl-
vinyl]benzoate.(**Compound 27**)</u>

Compound 27

Following General Procedure B, (Z)-4-[(5,5-dimethyl-8-phenyl-5,6-
dihydronaphthalen-2-yl)trimethylsilanylvinyl]benzyl alcohol was oxidized to give
the title compound (**Compound 27**). PNMR (300 MHz, CDCl3): δ 0.0 (s, 9 H),
1.50 (s, 6 H), 1.55 (t, J = Q7.4 Hz, 3 H), 2.51 (d, J = 4.8 Hz, 2 H), 4.52 (q, J = 7.4
Hz, 2 H),6.15 (t, J = 4.8 Hz, 1 H), 6.99 (d, J = 2.2 Hz, 1 H), 7.21 (dd, J = 2.2, 7.9
Hz, 1 H), 7.33 (s, 1 H),7.40-7.60 (m, 7 H), 8.12 (d, J = 8.3 Hz, 2 H).

<u>(Z)-4-[(5,5-Dimethyl-8-phenyl-5,6-dihydronaphthalen-2-yl)trimethylsilanylvinyl]-
benzoic acid.(**Compound 28**)</u>

Compound 28

Following General Procedure C, ethyl (Z)-4-[(5,5-dimethyl-8-phenyl-5,6-
dihydronaphthalen-2-yl)trimethylsilanylvinyl]benzoate was hydrolyzed to give the
title compound (**Compound 28**). PNMR (300 MHz, CDCl3): δ 0.13 (s, 9 H), 1.49

(s, 6 H), 2.50 (d, J = 4.9 Hz, 2 H), 6.13 (t, J = 4.9 Hz, 1 H), 6.97 (d, J = 1.7 Hz, 1 H), 7.20 (dd, J = 1.7, 7.9 Hz, 1 H), 7.31 (s, 1 H), 7.39-7.54 (m, 8 H), 8.17 (d, J = 8.4 Hz, 2 H).
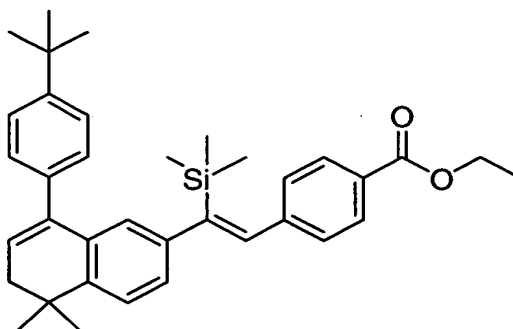
(Z)-4-{[8-(4-*tert*-Butylphenyl)-5,5-dimethyl-5,6-dihydronaphthalen-2-yl]-trimethylsilanylvinyl}benzyl Alcohol.**(Compound 29)**
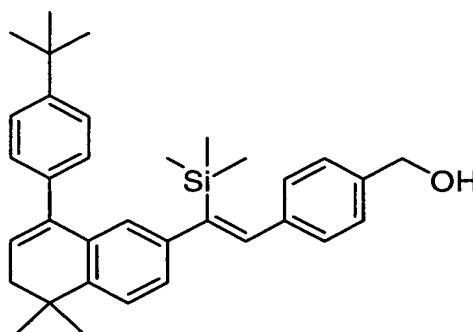
Compound 29

Following General Procedure A, 4-[(trimethylsilyl)ethynyl]benzyl *tert*-butyldiphenylsilyl ether and 6-bromo-4-(*tert*-butylphenyl)-1,1-dimethyl-1,2-dihydronaphthalene (which can be prepared by the procedure described in Klein, et al.,U.S. Patent 5,952,345) were coupled to give the title compound **(Compound 29)**. PNMR (300 MHz, CDCl3): δ 0.0 (s, 9 H), 1.48 (s, 6 H), 1.51 (s, 9 H), 2.49 (d, J = 4.8 Hz, 2 H), 4.82 (d, J = 4.8 Hz, 2 H), 6.13 (t, J = 4.8 Hz, 1 H), 7.00 (d, J = 1.7 Hz, 1 H), 7.22 (dd, J = 1.7, 7.9 Hz, 1 H), 7.30-7.50 (m, 8 H), 7.55 (d, J = 8.8 Hz, 2 H).
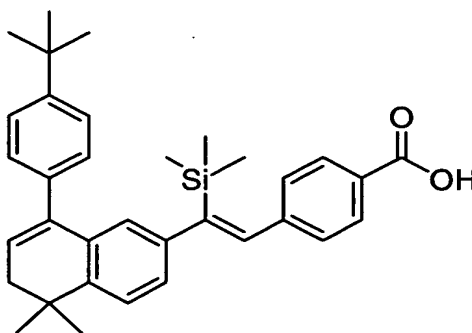
Ethyl (Z)-4-{[8-(4-*tert*-Butylphenyl)-5,5-dimethyl-5,6-dihydronaphthalen-2-yl]-trimethylsilanylvinyl}benzoate.**(Compound 30)**

(Compound 30)

Following General Procedure B, (Z)-4-{[8-(4-*tert*-butylphenyl)-5,5-dimethyl-5,6-dihydronaphthalen-2-yl]trimethylsilanylvinyl}benzyl alcohol was oxidized to give the title compound **(Compound 30)**. PNMR (300 MHz, CDCl3): δ 0.0 (s, 9 H), 1.49 (s, 15 H), 1.53 (t, *J* = 7.1 Hz, 3 H), 2.50 (d, *J* = 4.4 Hz, 2 H), 4.51 (q, *J* = 7.1 Hz, 2 H), 6.13 (t, *J* = 4.4 Hz, 1 H), 6.98 (d, *J* = 2.2 Hz, 1 H), 7.20 (dd, *J* = 2.2, 8.0 Hz, 1 H), 7.31 (s, 1 H), 7.40-7.54 (m, 7 H), 8.12 (d, *J* = 8.4 Hz, 2 H).

(Z)-4-{[8-(4-*tert*-Butylphenyl)-5,5-dimethyl-5,6-dihydronaphthalen-2-yl]-trimethylsilanylvinyl}benzoic Acid.(**Compound 31**)

(Compound 31)

Following General Procedure C, ethyl (Z)-4-{[8-(4-*tert*-butylphenyl)-5,5-dimethyl-5,6-dihydronaphthalen-2-yl]trimethylsilanylvinyl}-benzoate was hydrolyzed to give the title compound (Compound 31). PNMR (300 MHz, CDCl3): δ 0.0 (s, 9 H), 1.49 (s, 15 H), 2.50 (d, J = 4.8 Hz, 2 H), 6.13 (t, J = 4.8 Hz, 1 H), 6.96 (d, J = 2.2 Hz, 1 H), 7.20 (dd, J = 2.2, 7.9 Hz, 1 H), 7.31 (s, 1 H), 7.44-7.54 (m, 7 H), 8.18 (d, J = 8.4 Hz, 2 H).
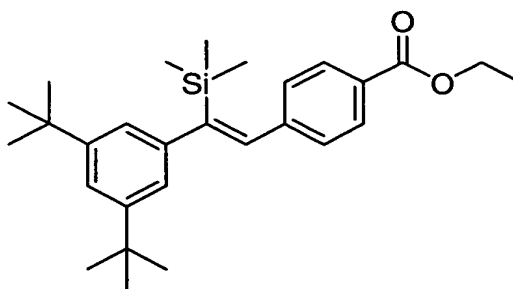
(Z)-4-[2-(3,5-Di-tert-butylphenyl)-2-trimethylsilanylvinyl]benzyl alcohol. (Compound 32)
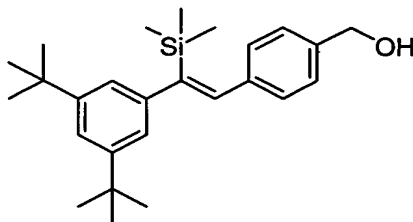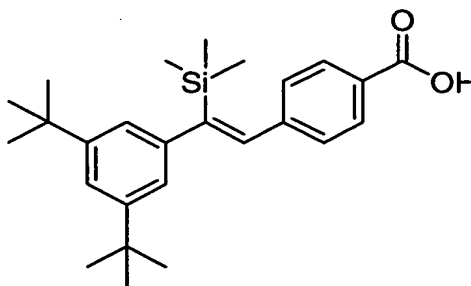

(Compound 32)

Following General Procedure A, 4-[(trimethylsilyl)ethynyl]benzyl *tert*-butyldiphenylsilyl ether and 1-bromo-3,5-Di-*tert*-butylbenzene (which can be prepared by the procedure described in Komen and Bickel *Synth. Commun*, 1996, 26, 1693-1698) were coupled to give the title compound (Compound 32). PNMR (300 MHz, CDCl3) δ 7.40 (s, 4H), 7.33 (s, 2H), 7.08 (s, 1H), 7.07(s, 1H), 4.78 (d, J=5.9 Hz, 1H), 1.41 (s, 18H), 0.00 (s, 9H).

Ethyl 4-[2-(3,5-Di-tert-butylphenyl)-2-trimethylsilanylvinyl]benzoate.(Compound 33)

Compound 33

Following General Procedure B, (Z)-4-[2-(3,5-Di-tert-butylphenyl)-2-trimethylsilanylvinyl]benzyl alcohol was oxidized to give the title compound **(Compound 33)**. PNMR (300MHz, CDCl₃) δ 8.08 (d, J=8.4 Hz, 2H), 7.46 (d, J=8.5 Hz, 2H), 7.32 (m, 2H), 7.07 (s, 1H), 7.06 (s, 1H), 4.45 (q, J=7.2 Hz, 2H), 1.47 (t, J=7.2 Hz, 3H), 1.41 (s, 18H), 0.00 (s, 9H).

4-[2-(3,5-Di-*tert*-butylphenyl)-2-trimethylsilanylvinyl]benzoic Acid. **(Compound 34)**

Compound 34

Following General Procedure C, ethyl 4-[2-(3,5-Di-tert-butylphenyl)-2-trimethylsilanylvinyl]-benzoate was hydrolyzed to give the title compound **(Compound 34)**. PNMR (300 MHz, acetone-d₆) δ 8.09 (d, J=8.2 Hz, 2H), 7.55 (d, J=7.9 Hz, 2H), 7.39 (m, 2H), 7.13 (s, 1H), 7.14 (s, 1H), 1.39 (s, 18H), 0.00 (s, 9H).

The examples set forth herein are meant to be illustrative only, and are not intended to limit the scope of the invention, which should be defined solely with reference to the claims that conclude this specification.